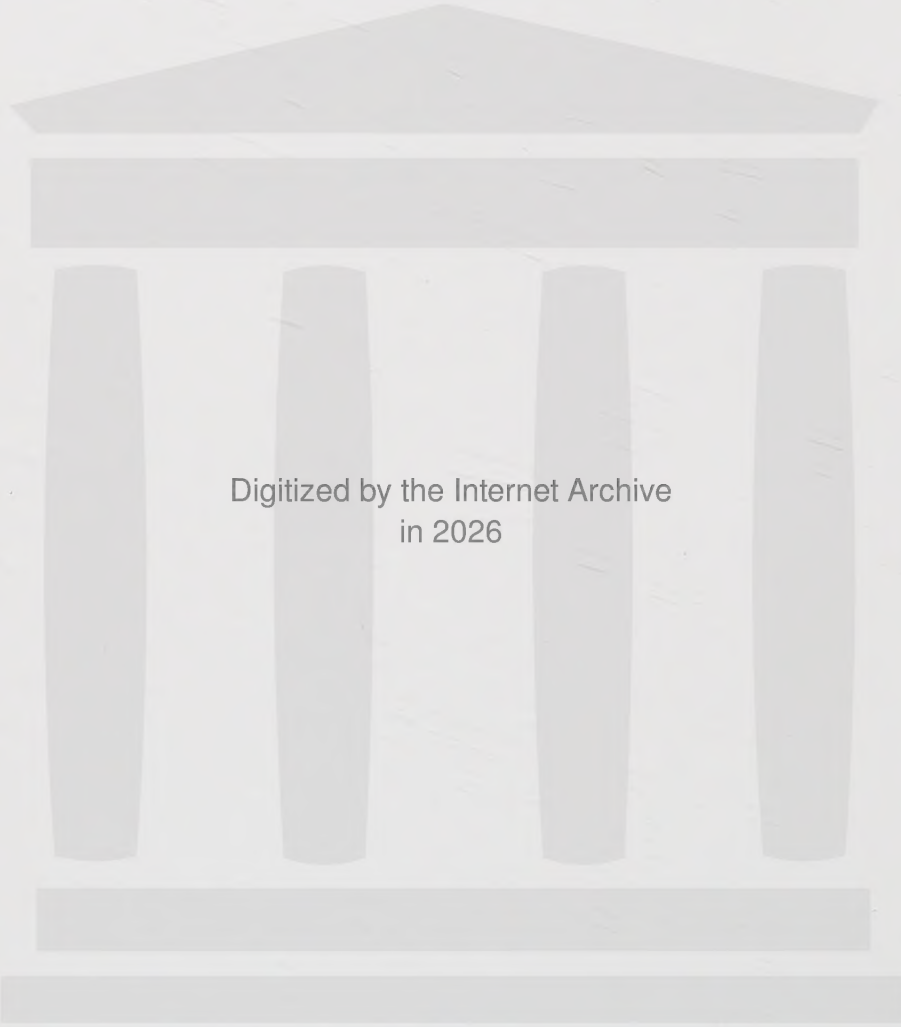


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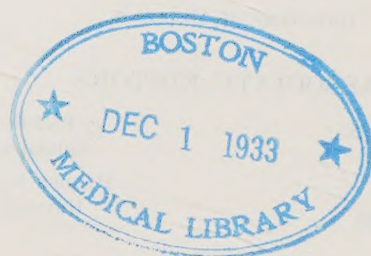
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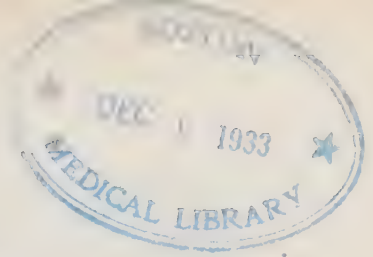
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Howard W. Schulte



HERMANN VON WECHLINGER SCHULTE

1876-1932

Dr. Hermann von Wechlinger Schulte, fifty-six, professor of anatomy and dean of the Creighton University School of Medicine, died on July 13th, at his home in Omaha, Nebraska. Death came as the result of an acute heart attack after a critical 2-week illness with gall bladder infection. Previous to this complication he had been in poor health for 3 years with asthma and intermittent cardiac disturbances.

Doctor Schulte, the son of an Episcopal clergyman, was born in Utica, New York, August 9, 1876. He was educated at St. Paul's, Concord, New Hampshire, and at Trinity College, Connecticut, where he received his B.A. degree in 1897. Possessing a splendid training in the classical studies, especially history, literature, and romance languages, he was eminently fitted to follow his father's successful work in the ministry, but chose instead a career in medicine, guided thereto by the wish of his mother. He was graduated with high honors as a Doctor of Medicine at Columbia University in 1902. After an internship rich in clinical experiences he entered the first stage of his public career as a teacher of anatomy under the able and inspiring leadership of Dr. George S. Huntington. From 1904 to 1917, the anatomical halls of Columbia were permeated with the enthusiastic spirit of this young anatomist, who, like the great Huntington, not only taught effectively and in master fashion, but also did brilliant research. The older anatomists well remember the chivalry and intellectual perspicuity displayed by Doctor Schulte during his discussion of anatomical papers at the yearly meetings of the American Association of Anatomists, his stand on the origin of lymphatics and endothelium being his most salient defense.

Suddenly, and to the great surprise of many of his colleagues, Doctor Schulte accepted the call to a professorship of anatomy at Creighton University. Junior dean for 2 years, he, in 1919, was appointed dean of the medical school, a position which he held until his recent untimely death.

It was at Creighton University that Schulte demonstrated his leadership in medical education. By example and exhortation he imparted to the minds of his students and graduates in the clinical fields the conviction that the justification and purpose of a medical school was not only its artistry and science, but primarily its service and benefit to the community. Much as he relished his anatomical research, he purposely caused it to be supervised by research in the social sciences, because he felt that the latter could most effectively give to the medical profession its necessary sociologic consciousness and adjust it to the ever changing conditions of practice. In Schulte's opinion the time was fast approaching when a radical change would be made in the business end of medicine. Since, under present conditions, the only patients who could afford the best of medical treatment were the very rich or the poor who were given every consideration of charity, Schulte contended that the vast majority of people now neglecting or only partially attending to their ailments should be taken care of by State medicine or group insurance.

As a result of his social studies and activities, Doctor Schulte held many civic posts. To list but a few, he was director of the Omaha Chamber of Commerce for 8 years, he was chairman of the Health Committee, vice-president of the Nebraska State Conference for Social Work, trustee of the Omaha Orthopedic Society, and chairman of the Nebraska Review of the White House Conference on Child Health and Protection.

As an academy officer Schulte served on the executive committee of the American Association of Anatomists, was vice-president of the New York Academy of Science, and president of the Nebraska Academy of Science. He was a member of many scientific societies, including the National Tuberculosis

Association, The American Academy of Politics and Social Sciences, and the United States Public Health Association.

It was my good fortune to be associated with Doctor Schulte at Creighton University as associate professor of anatomy for 2 years. As a teacher his didactic lectures were highly informative, inspirational, and provocative to independent thinking and study. Because of his exceptional mastery of the nervous system, it was both an intellectual treat and a feat to follow his delineation and description of the origin and termination of fiber tracts, his greatest delight being the presentation of the anatomy underlying the bottom of the acusticus internus, to wit, the auditory nerve, its abutment and its branches. Once having heard this lecture, one waited in suspense for that part of it dealing with the tractus spiralis foraminosus. Who could ever forget the genius of Schulte after hearing his description of the ontogeny and phylogeny of the thalamus, its relay pathways giving affective tone to sensation, its lesions as compared with those of the globus pallidus? If neuro-anatomy was Schulte's forte, his knowledge of gross, microscopic, and developmental anatomy was almost as extensive. I heard him lecture on the humerus for nearly 2 hours, the bone and chalk being his only support. His description of the temporal bone was a masterpiece of minutiae and was never completed in a single hour session.

To my repeated question, "How did you ever learn all this anatomy?" Schulte made the succinct reply, "Cheer up, young man, it takes 20 years to become an anatomist." What a loss to teachers of anatomy that Schulte did not write the book on anatomy which he had in mind and which he would have written had he retained his one time vigorous health. I have abundant stenographic notes of Schulte's lectures on the nervous system and feel that they should be published, for his reading was wide and his investigations were manifold and thorough. As a man, Schulte excelled in pedagogy and psychology. The task of dean is not always inducive to popularity, yet he was voted again and again the most popular member of the faculty. He loved students and was loved by them. The door of his administrative office was always

open, for he believed in youth despite its present veneer of cynicism and sophistication. As a result both junior and senior students never felt adrift, for under his tutelage they learned how to stand alone in study, clinical skill, and judgment. If a student failed in his medical studies, it was due solely to an unremedial defect in his preliminary education. Inspired by him, graduates went forth to be not the least socially minded of any group of cultured men as is usually the case with physicians to-day, but to assume their share and effort in the advancement of the related humanities.

Schulte had thousands of friends, yet one had to know him intimately to be his bosom friend. Being one of these, I knew him as he was, a philosopher with a mind that should have recorded its struggles, triumphs, and ingenious welding of human adversities. Traces of sorrow and melancholy could often be discerned in his tête-a-tête discussions on the philosophy of life, for he was an ardent student of Plato and Kant, Darwin, Huxley, Spencer, Hamilton, and Samuel Butler. Far from being a prey to intellectual grief, he enhanced his thoughts and conversations by relating a piquant anecdote, a pleasant remembrance, some witty souvenir of a life that was rich in human experience. Often he would cite a stanza or two of poetry to which I reacted as did Juvenal: *Semper ego auditor tantum, nunquam ne reponam.*

Schulte's scientific achievements in anatomical research, much of which was linked with the American Museum of Natural History, constitute outstanding contributions in the annals of American anatomy. His publications comprise such diverse subjects as the anatomy of whales, lumbar vertebrae of *Scutisorex*, the venous system of marsupials, the hydrodynamic factors involved in the organization of the venous return in monotremes, the development of the neuraxis, fusion of cardiac anlage, and formation of the cardiac loop, the development of the great veins and the hepatic circulation in the cat, and the histogenesis of the salivary glands. All works are admirably written, for Schulte had an unusual command of the English language. His wording reached its greatest display in his description of the temporal and squamous bone.

Schulte was the first to give a complete description of the skull of *Kogia breviceps*, the chief osteological features of this little known whale being the participation of the maxillae in the wall of the cranial cavity, the suppression of both nasals, the complete reduction of the suborbital bar of the malar, and the development of the midfrontal crest. In two other beautifully illustrated monographs Schulte describes the skeletal muscles of *Kogia* as compared with conditions in other odontocetes and mystacetes and gives a detailed account of the visceral anatomy of this animal. Schulte's major work on whales is a large monograph on the anatomy of the Sei whale (*Balaenoptera borealis*) a foetal specimen of which was taken by Roy C. Andrews at Aikawa, Japan, 1910, stored in the American Museum of Natural History, and graciously submitted to Schulte for analysis and anatomic description.

In his work on vasculogenesis, Schulte found no logical grounds for assigning to endothelium and blood the value of a fourth germ layer, the angioblast of His and Minot. By reconstructions he demonstrated endothelium to be a derivative of mesenchyme from which it arises diffusely, throughout ontogeny, by a multitude of separate anlagen. He supported the adaptive theory of endothelium for, in his opinion, "endothelium owes its presence to hydrodynamic forces and when these cease to operate, endothelium disappears as endothelium and reverts to mesenchyme or gives rise to products indistinguishable from those of mesenchyme."

By reconstructions Schulte showed that in the development of the heart the median angiocytes (groups of mesenchymal cells) play an important role in the coalescence of the endothelial anlagen analogous to that of the middle plate in the fusion of the myoepicardial mantles.

In his work on hepatic circulation, Schulte ascertained that there is an independent formation of capillaries in the septum transversum in the sense that a considerable portion of the hepatic vessels are formed before and independent of the resolution of the omphalo-mesenteric and umbilical veins into sinusoids.

The monograph on the development of the neuraxis in the cat by Schulte and Tilney, won Schulte a renown as a

neurologist. By carefully made reconstructions it was shown that in the cat the ganglionic crest is a derivative of the neural tube—a view which acquires an antecedent probability from the occasional retention of the afferent ganglia cells in the neuraxis of the adult, as the *Amphioxus* and teleosts, and in the mesencephalic root of the trigeminus of mammals. Since ganglionic elements are included in the wall of the neural tube at the time of its separation from the ectoderm, it was contended that in more cephalic portions of the neural tube where ganglia were not formed, the ganglionic elements must form a permanent constituent of the wall of the brain. In short, the ganglia of the cranial nerves were wholly derived from the neuraxis without the participation of the somatic ectoderm. The authors state that “The analysis of the prosencephalon is not to be attempted in terms of the basal and alar plates alone, as has been customary since His, but must include a dorsal and ectal strip equivalent to the ganglionic crest along its convexity, and this must include at least as much of the brain wall as lies ectal to the optic sulcus.” Accordingly, the optic vesicle and the ectoptic structures must be considered of ganglionic equivalency. Regarding the composition of the encephalon in terms of basal and alar plates, Schulte and Tilney showed that the sulcus of *Monro* is not a continuation of the sulcus *limitans*. The latter ceases to give evidence of its existence immediately in front of the oculomotor nucleus, this being the most cephalic structure by which its derivatives can be assigned to the basal plate.

How the memories of his work on the anatomy of whales lingered with Schulte even as a dean became apparent to me one morning when he showed me a telegram from the American Museum of Natural History informing him that a young unknown whale had been taken and that the authorities of the museum wanted him to do the anatomy. “What an opportunity and how I would like to go” said Schulte, but he remained at his post. It is well that he did, for the trail hewn out by him in modern medical education will lead the physician to individual and social betterment.

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CHARACTERISTICS OF THE GOLGI APPARATUS IN THE DIFFERENT TYPES OF CELLS OF THE ANTERIOR HYPOPHYSIS

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ONE PLATE (NINE FIGURES)

Although numerous studies have been made on the finer structure of the hypophysis cerebri, our knowledge is still far from complete. The lineage of the several varieties of cells contained in the anterior lobe, the relations of these cells to each other, and their relations to the hormone or hormones now known to be elaborated by this lobe, remain largely matters of conjecture.

In an attempt to throw light upon some of these relationships, a cytological study of the hypophysis of the cat was undertaken a number of years ago. A report has been made on the Golgi apparatus, the mitochondria, and the process of colloid formation of the pars tuberalis (Atwell, '29 b). It has seemed desirable to determine whether distinctive characteristics other than specific granules are possessed by the several varieties of cells of the pars anterior. Early it was discovered that the Golgi apparatus presents notable differences in size, density, and position in the different cell types of this lobe.¹ The present paper treats in more detail of the characteristics of the Golgi apparatus in the acidophile, basophile, and chromophobe cells of the anterior lobe of the cat's hypophysis, as revealed by study and measurement of numerous additional preparations.

¹The author's preparations illustrating these and other features have been demonstrated to the American Association of Anatomists on three occasions—at Buffalo in 1924; Rochester, 1929, and Chicago, 1931. A short description, with measurements, has been given in abstract form (Atwell, '29 a).

As material for this and certain related studies the pituitaries of eighty cats have been preserved. Except when it was desired to remove the gland for weighing, fixation was done by vascular injection. Approximately two-thirds of the glands have been sectioned serially. The remainder have been fixed and stained by various cytological techniques. Among these the Da Fano modification of Cajal's silver method early proved successful in demonstrating the Golgi apparatus. It was found possible to follow this with nuclear and cytoplasmic stains to demonstrate the cell varieties. Weigert's hematoxylin and acid fuchsin differentiated with picric acid or dilute picrofuchsin served to distinguish the acidophile cells from the other varieties, but did not differentiate among the latter. Mallory's connective tissue stain as well as various combinations of its components proved valuable as had been shown by Addison ('17) and by Rasmussen ('29).

The Da Fano method, followed by Heidenhain's azan stain, gave brilliant differentiation of the three types of cells and positive pictures of the Golgi apparatus. After formalin cobalt-nitrate fixation as used in the Da Fano technique, but omitting the silver treatment, even more brilliant cytoplasmic differentiation was obtained. The Golgi net, while presenting a negative picture in such a preparation, is very clearly shown, due to the intensity of the cytoplasmic coloration. It is highly desirable that sections be 4μ , or less, in thickness.

Among the osmic acid methods for the demonstration of the Golgi apparatus two have been tried with success. The Mann-Kopsch osmo-sublimate technique shows the Golgi apparatus well and when properly differentiated and followed by iron-alum-hematoxylin and acid fuchsin the mitochondria are stained black and the acidophile granules a deep red color. The Champy-Kull-Nassonov method also gives satisfactory results. If too much osmic is removed during differentiation the Golgi net becomes clear, thus rendering a negative picture of the apparatus. Such a preparation is scarcely less useful, however, than one presenting a positive image of

the apparatus, because of the intensity of the cytoplasmic staining. Especially brilliant is the differentiation of mitochondria and granules in the acidophile cells. Thus it is seen that both the Mann-Kopsch and the Champy-Kull-Nassonov

TABLE 1

Cats with hypophysis specially stained as utilized in the present study

NO.	SEX	WEIGHT	FIXATION	STAINING	REMARKS
		<i>grams</i>			
4	♀	'half-grown'	Bensley	Altmann	
11	♂	1375	Neutral formalin	Cajal	
14	♂	2901	Zenker-formol	Iron-alum-hematoxylin	
15	♂	3240	Zenker-formol	Iron-alum-hematoxylin	
17	♂	2230	Da Fano	Several methods	
45	♀	3250	Da Fano	Several methods	
46	♂	3295	Mann-Kopsch	Several methods	
47	♀	2530	Da Fano	Several methods	
48	♂	3210	Mann-Kopsch	Several methods	
49	♂	2877	Da Fano	Several methods	
50	♀	2653	Mann-Kopsch	Several methods	Postpartum or abortion
51	♂	2840	Da Fano	Heidenhain's azan	
52	♀	2635	Mann-Kopsch	Various	Embryos, 16 mm. C.R.
53	♀	3845	Da Fano	Heidenhain's azan	4 fetuses, 110 mm. C.R.
75	♂	1050	Da Fano	Heidenhain's azan	
76	♀	1100	Champy Formalin-	Kull-Nassonov	
77	♂	1162	Cobalt nitrate Formalin-	Various	
78	♀	1400	Cobalt nitrate Formalin-	Various	
79	♀	1200	Cobalt nitrate	Iron-alum-hematoxylin	

methods give clear differentiation of the Golgi net, mitochondria and cytoplasmic (secretory?) granules in the acidophile cells. The method recently described by Severinghaus ('32 b) also demonstrates these structures in a very beautiful manner.

OBSERVATIONS

Casual observation of an appropriately stained section shows that the Golgi apparatus is smaller in the acidophile cells than in the basophiles or in most of the chromophobes (pl. 1). This observation is fully sustained when careful measurements are made (table 2). It is found that in the acidophile cells the Golgi apparatus is almost invariably smaller than the nucleus of the cell. In the basophiles, and more particularly in the chromophobes, on the other hand, the apparatus may equal, or at times even exceed, the size of the nucleus.

Table 2 shows that the average size of the Golgi apparatus in the acidophile cells is $3.65\ \mu \times 2.58\ \mu$; in the basophiles, $5.48\ \mu \times 3.87\ \mu$, and in the chromophobes, $6.86\ \mu \times 5.62\ \mu$. It is true that an occasional chromophobe shows a net of the size characteristic of the acidophile cell. The number of these in the cat is relatively few. The possible significance of such cells will be discussed later in this paper. In selecting cells for measurement of the Golgi apparatus great care must be exercised to be as certain as possible that the structure is cut through its center. It has been the author's practice when measuring to proceed in an orderly fashion back and forth across a given section and to measure each cell of which the variety could be determined and in which, at the same time, the Golgi apparatus seemed to be cut through its center. In a section of any given thickness it is obvious that more of a small object may be included than of a larger one. Thus the measurements of the Golgi apparatus given are probably more accurate for the acidophiles than for the basophiles or the chromophobes. It is probable that the measurements for the latter err in being somewhat too small. If such is the case, the difference in the size of the apparatus is greater than is indicated by inspection of the tables.

In shape the Golgi net is usually more flattened in the acidophile cells (figs. 1 and 2) than in the chromophobes. This, however, is not invariably the case (fig. 5). In the basophile cell also the apparatus is flattened somewhat, its average

shorter dimension equaling nearly two-thirds of its longer, as in the case of the acidophiles (table 2). The larger net of the chromophobe cell is more nearly spherical. Its average lesser diameter equals over 80 per cent of its greater (table 2).

TABLE 2

Measurements in micra of the Golgi apparatus in the three principal cells of the anterior lobe of the hypophysis of the cat. Each number represents the mean of 10 measurements

	EOSINOPHILES	BASOPHILES	CHROMOPHOBES
Cat 53	3.60×2.56	5.00×3.44	5.80×5.30
Cat 51	3.66×2.57	6.16×4.31	7.12×4.98
Cat 75	3.70×2.62	5.28×3.87	7.65×6.59
Mean	3.65×2.58	5.48×3.87	6.86×5.62

The larger nets of the basophile and chromophobe cells often show that they are hollow spheres which sometimes, as seen in figure 3, are open on the side next to the nucleus. The cytoplasm constituting the core of the apparatus does not contain the characteristic granules of the chromophile cells. This is strikingly apparent in Champy-Kull-Nassonov preparations. The absence of specific granules in this central core is clearly shown by many other staining methods, i.e., Altmann, Mallory, and Heidenhain's azan. As seen in such preparations, this region, surrounded by the net, constitutes the 'macula' (compare Addison, '17 a).

Deineka ('12) has shown that in many cases the position of the centrosome and of the Golgi apparatus correspond (cells of Descemet's membrane, leucocytes, medullary cells of adrenal). It has been possible, by staining heavily with iron-alum-hematoxylin, and differentiating cautiously, to demonstrate the centrosome in the cells of the anterior lobe of the hypophysis. As shown in figure 6, it is present, at least sometimes, as a diplosome located in the interior of the Golgi apparatus.

The net is more dense in the acidophile cells than in either of the other two varieties. It has the loosest arrangement in

the chromophobes. In Da Fano preparations which show the apparatus clearly in the chromophobes, the net is sometimes so densely stained in the acidophiles that its internal structure cannot be made out. On the other hand, preparations ideally demonstrating the net in the acidophile cells may show the net only faintly stained in the chromophobes. When both are well shown in the same section, the net of the acidophile is usually much the denser of the two.

As regards position, one gains the impression, from examination of a large number of cells, that the apparatus nestles more closely to the nucleus in the acidophile cell than in the other varieties. This certainly is true when the acidophile cell is compared with the chromophobe, where an appreciable distance may intervene between the net and the nucleus (fig. 7).

The technique described by Cleveland and Wolfe ('32) has not been employed in this study. Consequently, no correlation can be made at this time between the types of cells seen in the anterior lobe of the cat's hypophysis and those described by these authors in the pituitary of the dog. It seems desirable, however, to note at this time a fourth variety of cell encountered especially in sections stained by the azan method. Present only in small numbers, this cell variety is very striking, due to the intensity with which it colors. The cell is rather small and somewhat flattened. Its staining is variable. Often the cytoplasm is an intense blue and the nucleus an equally intense fuchsin. The Golgi apparatus is small and dense. One gains the impression that this cell is at either the beginning or the end of a stage of great activity. It is possible that it shows the beginning of a degenerative phase, but this cannot be stated with certainty.

DISCUSSION

It seems very certain that the positive and negative pictures of the Golgi apparatus as described above represent the same structure. The correspondence in size, shape, and position is so close that little room is left for doubt. Prepara-

tions showing all degrees of impregnation of the Golgi apparatus have been examined, so that some cells show a positive and some a negative picture of the net in the same section. However, the two have never been observed in the same cell, as would be expected if the 'negative' (clear) picture represents a system of canals distinct from the Golgi net. Intra-cellular secretory canals have been observed and differentiated from the Golgi material in a number of externally-secreting glands (compare Dawson, '31, liver; Beams and King, '32 a and '32 b, salivary and gastric glands), but have not commonly been described for internally-secreting glands.

Concerning the difference in size of the 'macula' in the basophile and acidophile cells of the hypophysis of the albino rat, Addison ('17 a) said: "In the basophiles the macula is much larger than in the acidophiles." Addison hesitated at first to identify the 'macula' as the Golgi apparatus complex. This, however, he later was able to do (17 b).

De Beer ('26), while noting that the Golgi net varies in size in different cells of the hypophysis, did not make use of counterstains which would have enabled him to identify granular cell types.

In a short abstract the author (Atwell, '29 a) wrote: "In the acidophile cells (eosinophile, fuchsinophile) the reticular apparatus is constantly smaller and more compact in arrangement than in the basophiles or chromophobes."

Severinghaus ('32 a) states that in the rat "the specific type of Golgi which is present in the eosinophiles differs greatly from that found in the basophiles."

The present observations have shown that the largest and loosest Golgi nets are found in the chromophobe cells and the smallest and densest in the acidophiles, while in the basophiles the net is intermediate in size and density.

The significance of these facts as related to the lineage of the different varieties of cells is not yet entirely clear. Two views have prevailed regarding the relationships of the several cell types. One regards them as different phases of a

single secretory cycle. The other holds that the two types of chromophilic cells, "although originating from indistinguishable embryonic cells, develop on constantly diverging lines" (Bailey, '21).

Among those observers who hold to the latter or a related view there is no unanimity of opinion regarding the relation of the chromophile cells to the chromophobes. Perhaps the view most commonly accepted is that the chromophobes constitute a reservoir (as indicated by the name 'reserve cells') from which are differentiated both kinds of chromophile cells and back into which both varieties may return after losing their specific granules. In this connection the paper of Kraus ('14) should be consulted.

If condensation and reduction in size of the Golgi apparatus be taken as evidence of the maturing of secretory products, then the present observations could be construed as supporting the view that chromophobes differentiate into basophiles and these in turn into acidophiles in a single secretory cycle. To the author, however, the evidence for this view is inconclusive.

Addison ('17 a) has observed cells of the acidophile type which had lost most of their specific granules and which appeared to be returning to the reservoir of chromophobes.

Severinghaus ('32 a), working with the rat, has found among the chromophobes, cells with Golgi nets showing the differential characteristics of eosinophiles and basophiles. He points to the presence of these two Golgi types among the chromophobes "as evidence to substantiate the theory that basophiles and eosinophiles do not interchange in a secretory cycle, but are each related to specific chromophobe cells." Whether the last part of this conclusion is warranted seems questionable to the writer. It would be exceedingly difficult to prove that these different types of chromophobes maintain their identity throughout the so-called 'resting' phase. In the cat, as recorded on a previous page, the number of chromophobes with a Golgi net of the small condensed variety characteristic of the eosinophile cell is very small. In most

of the chromophobe cells the Golgi apparatus resembles that found in the basophiles more than it does that in the eosinophiles. As noted above, the average size of the net is even greater in the chromophobes than in the basophiles. This fact does not give support to the belief that the Golgi apparatus is necessarily static when once its characteristic size and shape are acquired.

What seems to be fully established, however, is that a criterion other than specific granule content has been found for distinguishing between the different cells of the anterior lobe. That these characteristic differences in the Golgi apparatus may be present before specific granules are developed or may persist after these granules have been lost seems clear. That the Golgi net does not revert to a less differentiated type after its character is once established is far from proved.

SUMMARY

The cells of the anterior lobe of the cat's hypophysis have been studied after preparation by a variety of staining methods. These include Da Fano's modification of the Cajal silver method for the Golgi apparatus followed by such stains as Mallory's connective-tissue stain or Heidenhain's azan; ManKopsch osmo-sublimate, and Champy-Kull-Nassonov. Some methods demonstrate Golgi apparatus, cytoplasmic granules, and mitochondria in the same preparation.

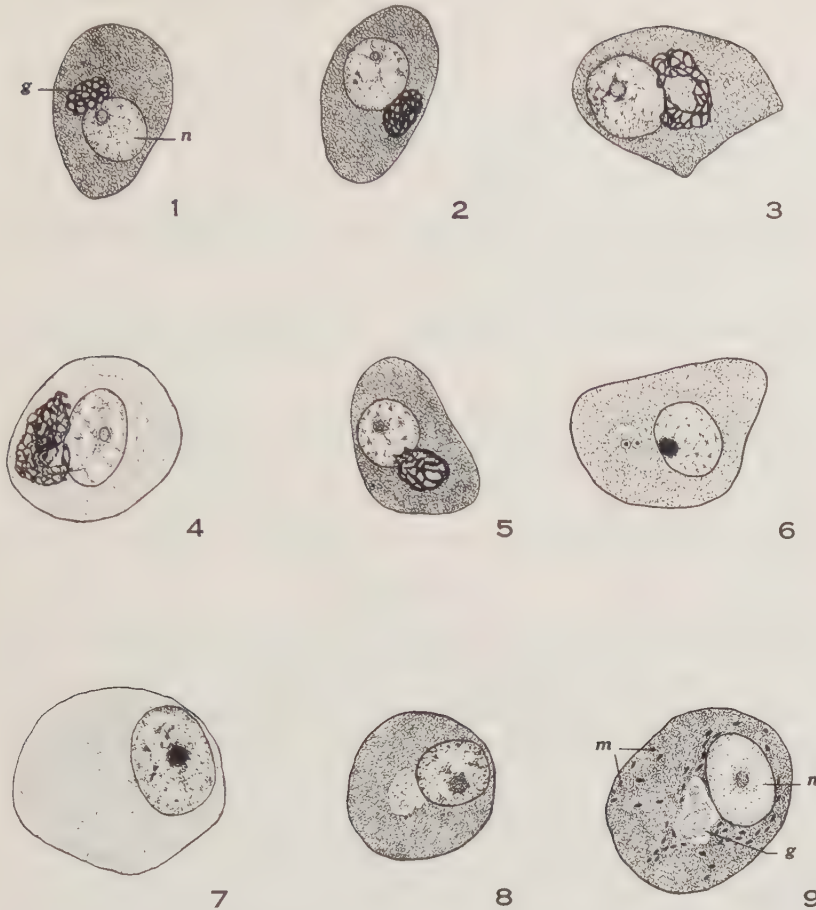
The Golgi apparatus is found to possess differential characteristics in the several varieties of cells of the anterior lobe. In the eosinophiles the net is small (averaging $3.65 \mu \times 2.58 \mu$), dense, somewhat flattened, and closely applied to the nucleus. In the basophiles the net is larger (averaging $5.48 \mu \times 3.87 \mu$) and looser. The chromophobes usually contain the largest nets (averaging $6.86 \mu \times 5.62 \mu$) which are commonly more spherical, more loosely arranged and more distant from the nucleus than in the chromophile varieties. Chromophobes containing the type of Golgi net characteristic of the eosinophiles have been observed, but are relatively few in number in the cat's hypophysis.

The centrosome, commonly as a diplosome, is found in the interior of the Golgi apparatus.

The possible significance of the above observations in relation to the lineage of the different varieties of cells is discussed.

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All figures were drawn with the Zeiss apochromatic $90\times$ oil-immersion objective, $20\times$ compensating ocular, and Spencer camera lucida. This gave a magnification at the table of 3300 diameters. In reproduction this has been reduced to 1650 diameters.

- 1 Eosinophile cell, cat 51. *n*, nucleus; *g*, Golgi apparatus.
- 2 Eosinophile cell, cat 53.
- 3 Basophile cell, cat 51.
- 4 Chromophobe cell, cat 53.
- 5 Eosinophile cell, cat 50.
- 6 Cell showing the centrosome, as a diplosome, in the interior of the Golgi apparatus, cat 77, iron-alum-hematoxylin stain. In this and the succeeding figures the Golgi net is seen as a 'negative' (clear) picture.
- 7 Chromophobe cell, cat 75.
- 8 Eosinophile cell, cat 75.
- 9 Eosinophile cell, cat 76. This preparation differentiates a 'negative' Golgi net, acidophile granules and mitochondria. *n*, nucleus; *g*, Golgi apparatus; *m*, mitochondria.

BONE STUDIES IN ULTRA-VIOLET LIGHT (I)¹

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ONE TEXT FIGURE AND THREE PLATES (TWELVE FIGURES)

INTRODUCTION

The various types of normal and pathological bone are differentiated most accurately by methods calculated to bring out the arrangement of the collagen fibers. The importance of fiber structure as a means of classifying bone has been stressed by many writers since the time of von Ebner (1874). Unfortunately, it is not always easy to demonstrate the fibers in bone. Many stains have been devised, but they are very capricious. The types of bone have also been demonstrated by the use of polarized light, but this shows only the gross arrangement of the fibers.

Dr. E. P. Fowler, Sr., suggested to us three years ago that the ultra-violet microscopy redeveloped by F. F. Lucas ('26) for the study of steel might be an ideal way to study bone. The so-called optical sections described by Lucas ('30) and by earlier writers would make it unnecessary to have the exceedingly thin sections required for the usual methods. Furthermore, since the ultra-violet work could be done on

¹ A part of the work on the ultimate structure of bone being done in connection with researches on otosclerosis under the auspices of the Department of Otolaryngology and the New York League for the Hard of Hearing. The sections used were prepared under funds from them; the photographic materials were supplied by the Department of Dental Histology.

unstained specimens, it would be possible to study the same sections in several different ways. Even absolutely untreated specimens of bone in normal saline could be studied when a method is perfected for cutting sections of undecalcified bone.

An ultra-violet microscope was put at our disposal through the courtesy of Prof. Charles F. Bödecker, of the Department of Dental Histology. We have found that photomicrographs of many types of bone can be secured and that fibers can be demonstrated in both stained and unstained sections of bone by the use of ultra-violet light.

In this paper we are presenting pictures of various types of bone, describing the exact technique used, and comparing it with other methods (pl. 1). Subsequent papers will deal with osteoid tissue, the so-called cartilage islands of the labyrinthine capsule, etc., as well as otosclerosis and other diseases of bone.

Since the apparatus was first perfected by Köhler ('04), only a few articles on the subject of ultra-violet photomicrography have appeared, but a variety of tissues have been studied, all with illuminating results. Von Shrötter ('06), on blood; Bödecker ('06), on teeth; Ernst and Wolbach ('06), on bacteria and fungi, were among the pioneers. In the past few years the method has been taken up again by Barnard ('20, '25), Lucas ('30), and Wyckoff with Ter Louw ('31). Gage ('32) has been advocating the use of various types of light for the study of tissues. However, in his ultra-violet work he employs longer wave-lengths and fluorescence rather than the selective absorption of ultra-violet rays. All of these workers agree that new and worth while observations can be made with ultra-violet light and emphasize that the technique is only slightly more difficult than the taking of ordinary photomicrographs. They also point out that wave-lengths of 280 μ and 275 μ in the ultra-violet have more than double the resolving power of visual light which has wave-lengths of 400 to 700 μ . Herein lies the main advantage of illuminating very small objects with ultra-violet light (Sabin, '06).

METHOD

The apparatus used by us (fig. 1) is of the type described and developed by Köhler while working in the Zeiss laboratories. It consists of a magnesium arc *d*, the light which passes through a slit. The image of the spark is focused upon quartz prisms *e* by a collimator

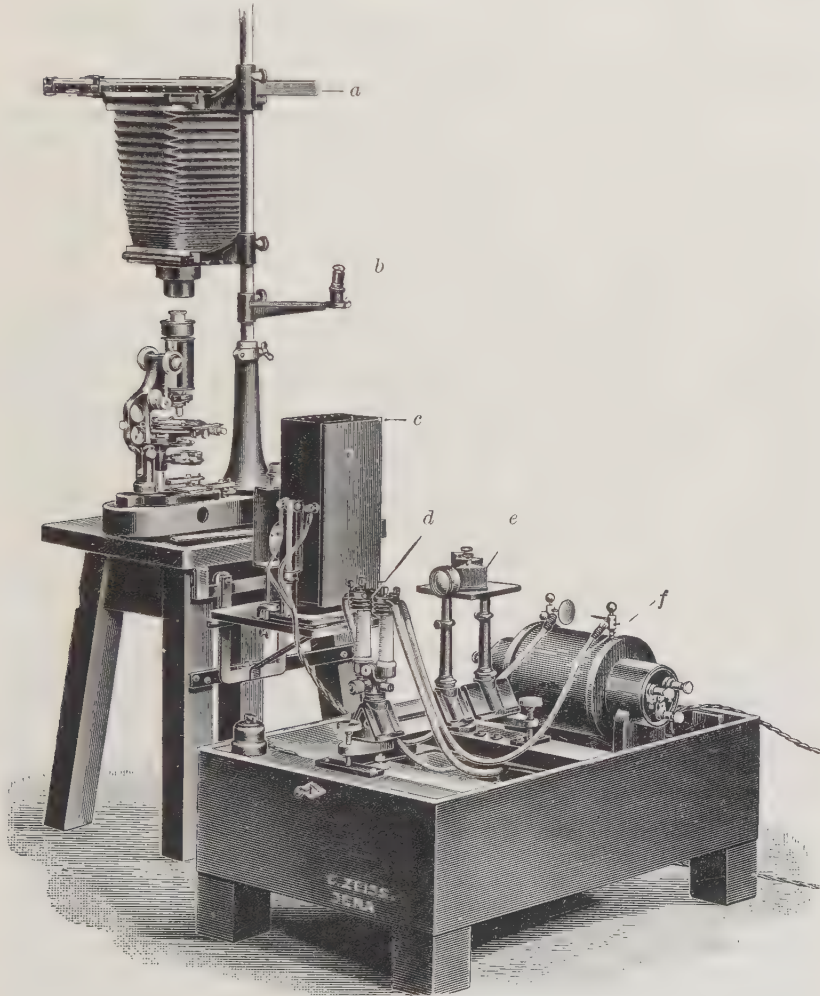


Fig. 1 Ultra-violet microscopy apparatus. *a*, sliding plate holder for taking several exposures. *b*, searcher eyepiece, containing fluorescent screen which gives hazy image of object to be photographed. *c*, mercury vapor lamp for finding field. *d*, magnesium or cadmium arc. *e*, quartz prisms. *f*, induction coil.

(also of quartz). After being broken up into a spectrum by the prisms, a second collimator focuses the desired line of the dispersed light upon a right angled prism, which reflects the light of this line only, upward into the microscope. The latter has a lens system (also of quartz) consisting of a substage condenser, an objective, and an ocular. Above the ocular is a simple camera bellows with a special sliding plate holder *a*.

The specimen is mounted on a quartz slide with a mixture of glycerin and water and covered by a thin quartz cover glass.

The field is found with artificial light, or with monochromatic light from a mercury vapor lamp *c* with a yellow or blue filter. The searcher eyepiece *b* is then swung into place, and by carefully focusing down with the diaphragm somewhat closed, a hazy image of the object to be photographed comes into view, if the section does not absorb too much of the light. The highest point at which this image seems to be in focus is now sought. (If no image is seen in the eyepiece, as is sometimes the case with thick, dense sections, the fine adjustment is turned downward, the number of revolutions found by experiment to be in the region of the correct focus.) Then the camera is swung into position, and seven different exposures taken, using the sliding plate holder provided by Zeiss *a*. It was found that starting at such a point and focusing downward, $5\ \mu$ at a time, is a satisfactory method for obtaining one picture which is in good focus. In this neighborhood seven more pictures are taken, this time changing the focus 1 or $2\ \mu$ for each exposure. At the point found to be in sharpest focus a 5×7 plate is taken. Ordinary process plates were used and the exposure varied from 15 seconds to 3 minutes, according to the thickness of the section and the magnification.

The ultra-violet band used in most of the work was the band from the magnesium arc which showed brightest on a uranium disc inserted in a carrier beneath the condenser. The latter was centered by closing the diaphragm and observing the light in a uranium disc placed on top of the tube of the microscope; the ocular, of course, being removed for this maneuver.

Since a second quartz spectograph was not available and the apparatus, as originally designed, is too crude for accurate analytical work, we have not as yet checked the assertion of other workers that the first sharp and very bright band after the visual spectrum is with a magnesium arc, the image of two lines with a wave-length of about $280\ \mu\mu$; and with cadmium, the line at $275\ \mu\mu$. It was found, however, that with this unmistakable bright band the best results were obtained.

The specimens which showed the fibers most clearly were prepared in the following manner:

Fixation, 10 days 10 per cent formalin with O.D. changes (room temperature).
Decalcification, 21 days in 1 per cent nitric acid in 10 per cent formalin (room temperature).

Na₂SO₄ for 48 hours.

Wash for 48 hours in running water.

50 per cent alcohol for 1 to 2 days.

80 per cent alcohol for 3 days.

95 per cent alcohol for 3 days.

Absolute alcohol for 1 day.

Ether—alcohol, equal parts, for 12 hours.

2 per cent parlodion (Du Pont) for 6 weeks, corked.

8 per cent parlodion (Du Pont) for 2 to 3 weeks, corked.

12 per cent parlodion (Du Pont) for 6 to 8 days, loosely covered.

Cut into blocks, mounted and cut at 16 μ , and stored on pieces of ordinary typewriter paper in 80 per cent alcohol.

OBSERVATIONS USING THIS METHOD

Bone of the type found in the cat's mandible and in the human temporal bone is particularly suited for ultra-violet photomicrography because the fibers are fairly coarse and of the parallel fiber type. This allows for a greater extent of their course being seen if they are photographed in the proper plane.

In ultra-violet photomicrographs of the mandible of an adult cat (6 mm. obj. 5x oc. mag. 220x) it was observed that most of the collagen fibers are laid down in parallel fashion about the blood vessels (fig. 2). Their arrangement is similar to that of the fibers described by Max Meyer ('27) in the temporal bone and by von Ebner (1875) in the long bones of birds. In the labyrinthine capsule, Max Meyer calls this type of bone 'fine fibered lamellarless marrow bone' or 'skein-like bone.'

Besides the parallel fibers, there are intermediate areas which appear as a conglomeration of fine dots with occasional short, fine fibers. These areas are evidently reticular or 'web-like bone' or are areas where the parallel fibers were cut transversely (fig. 12).

There are no areas in our specimens from the region of the incisors in the cat's mandible which show any of the typical 'shell bone' of Weidenreich. That is, there are no areas of the familiar lamellar systems characteristic of the long bones of the adult human body.

When the original negatives (mag. 220x) were *enlarged*, it was found that new detail appeared which could not be made out in the contact prints. In particular, besides the fibers described above, which absorb very little ultra-violet light, we were surprised to find a few fairly long-straight delicate fibers which seem to absorb much more ultra-violet light. These run more or less in the same direction as the collagen fibers, but appear black instead of white on the positives and resemble very closely fibers seen in the neighboring blood vessels (fig. 8). They are of the same size as canaliculi, but appear to be altogether too long and straight for canaliculi and they have no lacunar connections. It is possible that they are merely diffraction phenomena. Since nothing exactly like them has been demonstrated in other bones, their significance will have to be further studied.

With the very high power 1.7 mm. objective, using glycerin immersion for both the objective and the condenser, even finer fibers than those seen with the 6 mm. objective become evident (pl. 3), and the fibers seen by the 220x enlargement often turn out to be bundles of fibers. There are a few of the very fine fibers in the specimen from the cat's mandible (fig. 7), but there are many more in the capsular portions of the temporal bone (fig. 13).

Our photographs of lamellar bone have been made up entirely of these very fine fibers, if they showed any fiber structure at all. The fibers seem to be laid down so compactly in lamellar bone that good pictures of them cannot easily be obtained. Another difficulty is, that nothing but dots appear on the plate unless the fibers run parallel to the plane of the section; so that, although the lamellae may show up perfectly well as streaks of dots, the direction of the fibers is apparent only in those rare areas where the section was cut in a proper plane for them (fig. 9).

True reticular or 'web bone' such as that found in otosclerotic foci or in embryonal bones is characterized by the fact that very few parallel fibers can be made out in it. In ultra-violet light photomicrographs it appears as a number of

fine dots with here and there a few fibers cut in the plane of the section. The cells, of course, are of the stellate embryonal type instead of the adult more spindle-shaped type. The canaliculi run in all directions instead of running in certain planes as in parallel fiber bone and lamellar bone (fig. 11).

COMPARISON WITH OTHER METHODS

As a check on our observations, the actual sections studied by ultra-violet light were also studied with white light (fig. 3), with monochromatic light, with various bands in the ultra-violet, and with polarized light (fig. 4). They were then stained with hematoxylin and eosin (fig. 5), while nearby sections in the series were stained with silver.

The specimens prepared in the routine manner,² used in the Department of Pathology for the preparation of temporal bones gave the most satisfactory picture of bone fibers. A specimen of human rib fixed in Zenker's fluid and embedded in paraffin showed no trace of a fine fiber structure. Certain temporal bone specimens, which had been fixed too long in strong formalin, also failed to show the fibers.

When an unstained specimen is mounted in glycerin on a glass slide and studied in white light with a closed diaphragm, a distorted picture of the fibers present is obtained (fig. 3). This diffraction picture often suggests a parallel arrangement of the fibers, but shows none of the finer structure of the bone.

With ultra-violet light, since we have practically a point source, it seems to make no difference whether the diaphragm is open or closed, if the condenser is properly adjusted. If, however, the condenser is racked down too far, throwing the picture slightly out of focus, or if the light is not properly centered, lines of light interference are seen about such structures as lacunae and occasionally about a bundle of fibers. For example, although the canaliculi absorb ultra-violet to some extent, sharper pictures of them can be obtained by closing the diaphragm and racking down the condenser (fig. 10), but this is a slightly distorted picture. If one bears in mind its limitations, however, the method may be useful.

² See under METHOD.

When a glycerin preparation is studied by polarized light with the gypsum plate (red, first order), the groups of fibers which appeared to be almost parallel in white or ultra-violet light were found to be blue at one point on a revolving stage, yellow when the stage is revolved 90° from this point and red at all other points. This is the method of von Ebner (1874) for demonstrating parallel fibers in bone.

Certain areas in the preparations remained constantly red or contained very small spots which could be changed into yellow and blue by revolving the stage. These areas in ultra-violet photomicrographs showed many small dots and crossed lines and were therefore considered to be reticular bone or areas in which the fibers had been cut transversely. From the observation of the specimens in polarized light, we can be certain that we were correct in our classification of the various types of bone as seen in ultra-violet photomicrographs.

Agfa color plates can be made to record the appearance of tissues in polarized light with a gypsum plate, but the conventional black and white picture without the use of a gypsum plate gives a good idea of the appearance of bone in polarized light (fig. 4).

Experimentation with visual monochromatic light, either from a mercury arc with filters or the blue light from the spectrum of the magnesium arc, demonstrated nothing unusual. The use of long ultra-violet waves was unsatisfactory, because the tissue did not seem to absorb the light from them. With short wave-lengths, too little light was transmitted through the bone for sharp pictures to be obtained.

Short wave-length ultra-violet rays are selectively absorbed by unstained preparations just as visual light is absorbed by the dyes in stained preparations. The $280\text{ }\mu\mu$ band from the magnesium arc produces the best pictures of bone because the wave-length is short enough for some absorption by the nuclei, canaliculi, and certain fibers, and at the same time the light source is intense enough to affect the plate.

As is well known, hematoxylin-eosin staining of bone produces excellent preparations for the visualization of such structures as the nuclei of the cells, the outlines of lacunae and canaliculi, the cement and 'ansatz' lines, osteoid, etc. But with white light, the arrangement of the fibers and the type of the bone can only be inferred from the shape of the lacunae and the arrangement of the canaliculi. Weak hematoxylin-eosin staining, incidentally, does not materially affect the picture of the fibers obtained with ultra-violet light.

Silver impregnation methods, such as the various modifications of Bielschowsky's technique (Maresch, Laidlaw, etc.), bring out a fiber structure in bone, but show no other detail. Furthermore, it is quite difficult to obtain silver stained specimens which do not have the fibers matted together. When the fibers are far apart, as in reticular bone, the method is fairly satisfactory, but with parallel fiber bone and with lamellar bone, what appears as a single fiber may well be a bundle of fibers.

With the methyl-violet stain of Weigert much finer fibers are apparent than with silver stains, but, unfortunately, most of them are so delicate that even with an oil immersion objective they can barely be seen. Ordinary photomicrographs of these specimens are unsatisfactory, because the resolving power of even the best obtainable glass lenses is not great enough to bring out the finer fibers sharply.

The expense, the tedious 'blind focusing,' and the necessity of making all observations on a photographic plate are drawbacks to ultra-violet photomicrography. However, the increased resolving power attendant upon its use and the possibility of studying unstained preparations, are enough to counterbalance these disadvantages.

SUMMARY

It has been found possible to differentiate the various types of bone according to their fiber structure as demonstrated by ultra-violet photomicrography.

The mandible of an adult cat showed two kinds of fibers; rather coarse ones which absorb comparatively little ultra-violet light of $280\text{ }\mu\mu$, and fine sparse ones which do absorb these rays. The fibers in the cat's jaw and fibers in the capsule of the human temporal bone were found to be easiest to photograph, because the fibers are relatively coarse and are laid down in a nearly parallel fashion. The method is, however, applicable to the study of long bones and pathological bones.

The method is more certain than silver stains. It gives more information than the use of polarized light, because it shows the individual fibers. The bone cells, lacunae and canaliculi appear in the pictures. This is not so with other methods for showing the fiber structure of bone.

The selective absorption of ultra-violet and the high resolution attendant with its use, make it superior to white or monochromatic visual light for the study of bone. The $280\text{ }\mu\mu$ band from the magnesium arc, because of its intensity, produces better pictures of bone than other bands in the ultra-violet.

It is concluded that ultra-violet photomicrography is a worth while tool for the study of bone.

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PLATE 1

EXPLANATION OF FIGURES

2 Ultra-violet photomicrograph of a typical area in the mandible of an adult cat. The specimen was fixed in formalin, embedded in celloidin, and cut in cross section at about 16μ . Note the size and arrangement of the bone fibers and compare figures 3, 4, and 5. The structure at the bottom of the photograph is the wall of a small blood vessel cut longitudinally. (Zeiss monochromat. 6 mm. objective, 5x quartz ocular, 30 cm. camera length. Mag., approx., $\times 220$.)

3 Same unstained specimen shown in figure 2, photographed in white light with the condenser racked down and the diaphragm closed. Note diffraction. (Leitz, apochromat. 8 mm. objective, Periplan. 8x ocular, 30 cm. camera length. Mag., approx., $\times 210$.)

4 Same unstained specimen mounted in glycerin on glass and photographed through crossed nicols (no gypsum plate). The white areas are fibers running in one direction. All areas in which the fibers run in a different direction are black or gray. By revolving the specimen on a stage, other areas of parallel fibers can be made out. Those areas which remain black through 360° are reticulated fibers or fibers which run in the optical axis of the microscope. Areas containing fibers which are not double-refractive, such as the blood vessel wall in the lower portion of the picture, also remain black. (Leitz, apochromat. 8 mm. objective, Periplan. 8x ocular, 30 cm. camera length, Zeiss polarizing attachments. Mag., approx., $\times 210$.)

5 The same section and field shown in figures 2, 3, and 4 stained with hematoxylin and eosin, mounted in glycerin and photographed with white light, using a green filter. The wall of the blood vessel at the bottom has from much handling broken away from the bone. (Leitz, apochromat. 8 mm. objective, Periplan. 8x ocular, 33 cm. camera length. Mag., approx., $\times 220$.)



Fig. 2 Ultra-violet light.

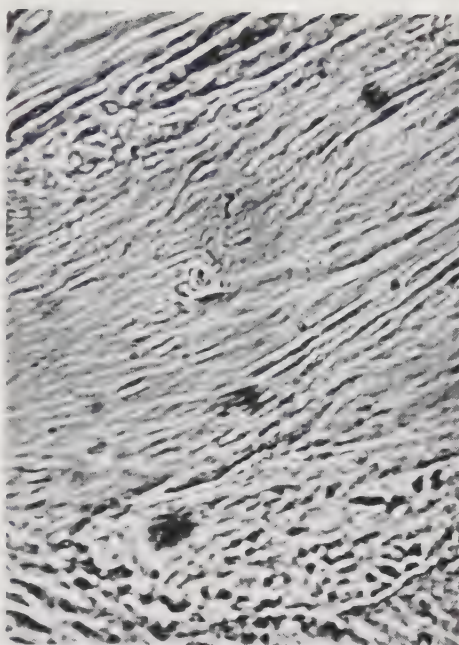


Fig. 3 White light.



Fig. 4 Polarized light.



Fig. 5 H and E stain, white light.

PLATE 2

EXPLANATION OF FIGURES

6 High power of an unstained section of the jaw of an adult cat. Photograph taken with white light. (Zeiss, 2 mm. oil immersion, 6x ocular. Mag., approx., $\times 1400$.)

7 Same field as figure 6, photographed with ultra-violet light. (Zeiss, 1.7 mm. glycerin immersion monochromat. 5x quartz ocular, 30 cm. camera length. Mag., approx., $\times 1400$.)

8 Ultra-violet photomicrograph of bone from jaw of adult cat, showing parallel fiber bone. Note that there are white and black fibers and that the black fibers in the bone resemble the tiny black fibers in the wall of the blood vessel at the right. (4.7x enlargement of a plate taken with Zeiss, 6 mm. monochromat. 5x quartz ocular, 30 cm. camera length. Mag., approx., $\times 1000$.)

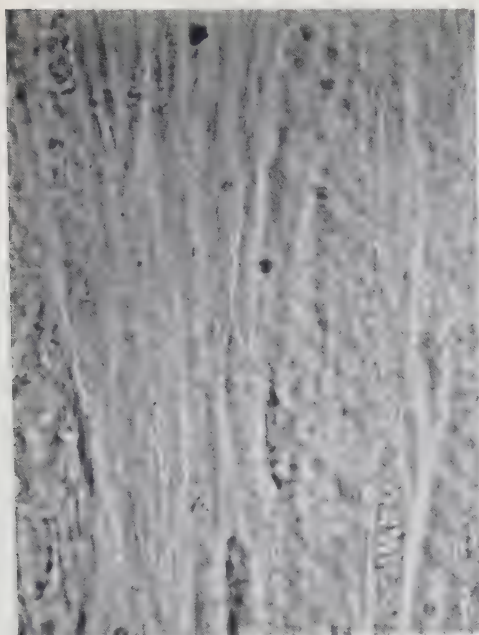


Fig. 6 Oil immersion, using white light



Fig. 7 Glycerin immersion, using ultra-violet light.

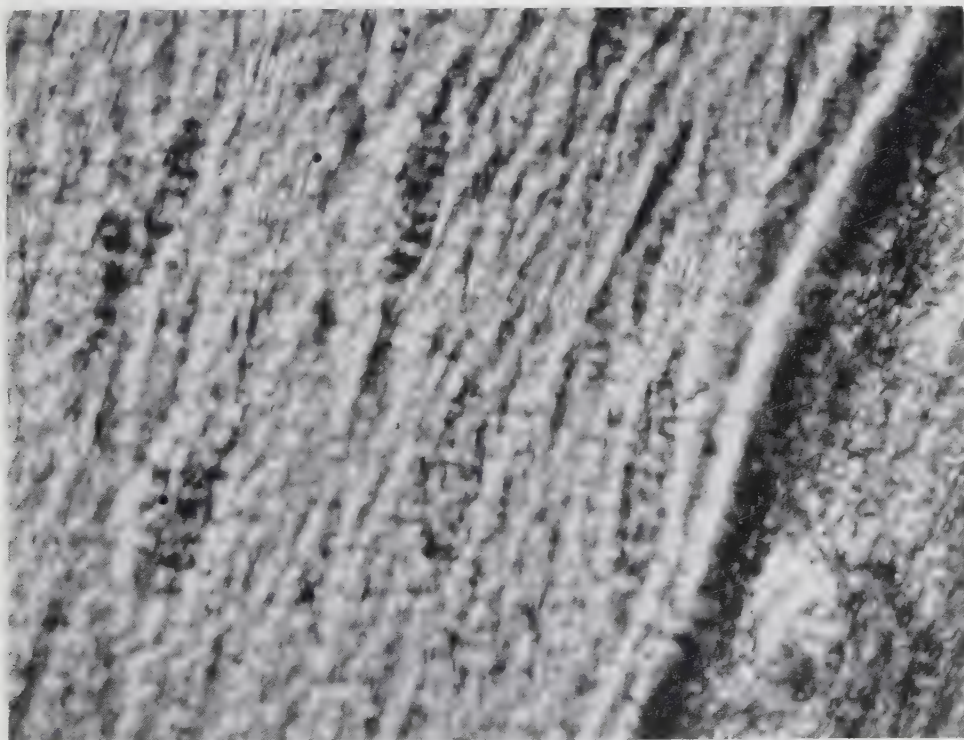


Fig. 8 Photographic enlargement of the U V plate.

PLATE 3

EXPLANATION OF FIGURES

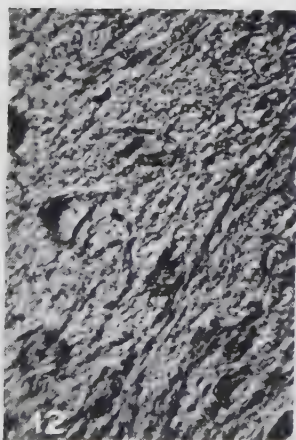
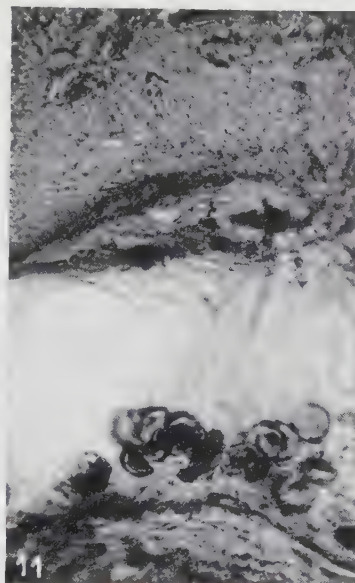
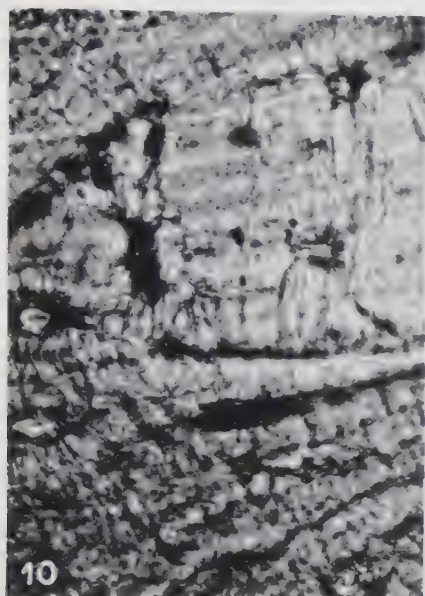
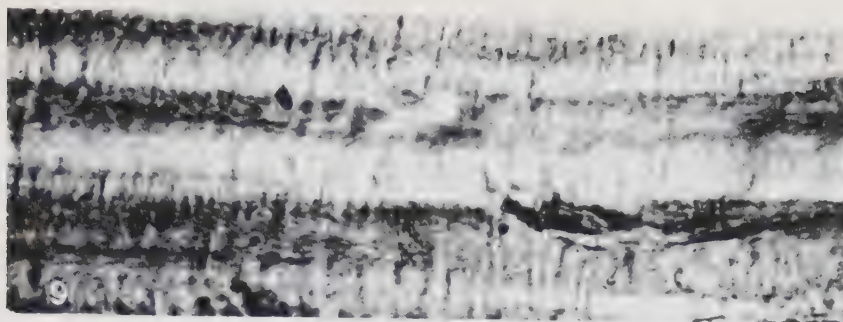
9 Lamellar bone from human mandible. Note the fine brush of fibers in those lamellae in which the fibers were cut in the plane of the section. The thick cross striations are canaliculi. (1.3x enlargement of a plate taken with a Zeiss 1.7 mm. monochromat. 5x quartz ocular, 30 cm. camera length. Mag., approx., $\times 1800$.)

10 Lamellar bone from the periostially formed portion of the human labyrinthine capsule; diaphragm closed and condenser racked down. Note lines of light interference about lacunae. Most of the fibers seem to be cut transversely. (Zeiss, 1.7 mm. monochromat. 5x quartz ocular, 30 cm. camera length. Mag., approx., $\times 1400$.)

11 Short fiber reticular bone from the middle of an otosclerotic area. Note the red cells in the marrow cavity, which runs transversely across the middle of the picture. Some of these are in focus and some, those which show up as concentric rings, are out of focus. Note that there are a few bundles of parallel fibers along the edge of the marrow space. (Lens system same as fig. 10. Mag., approx., $\times 1400$.)

12 Bone from the human labyrinthine capsule. Most of the fibers are cut transversely and are seen as dots. There are a few fibers which are longitudinal to the plane of this section. (Lens system same as fig. 10. Mag., approx., $\times 1400$.)

13 Parallel fiber bone in the human labyrinthine capsule. (Lens system same as fig. 10. Mag., approx., $\times 1400$.)



Various types of bone in ultra-violet light.

POTENCIES OF TRANSVERSE LEVELS OF THE CHICK BLASTODERM IN THE DEFINITIVE- STREAK STAGE

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ONE TEXT FIGURE AND TWO PLATES (FOUR FIGURES)

INTRODUCTION

This report is a continuation of earlier work which attempts to analyze the significance of the primitive streak and the rôle of its constituent parts in the development of the chick embryo by means of transplanting various regions of the blastoderm to the chorio-allantoic membrane. Previously it was shown that the node region of the blastoderm has a much greater capacity for differentiation than the more posterior levels of the primitive streak. Since the comparative potentiality of the different levels of the node and streak was not fully determined, the present report deals primarily with that problem. At the same time it seemed desirable to fix more exactly the boundaries of the node as a center of development, and with this in mind, careful notations and measurements have been made of the parts transplanted.

Methods other than that of chorio-allantoic grafting have been used in analyzing the developmental physiology of the chick. Wetzel ('29) has determined the prospective values of different areas of the early blastoderm by means of applying vital stains to certain regions and following the subsequent fate of the stained cells. Using the chorio-allantoic grafting method, we have transplanted isolated pieces corresponding to those areas stained by Wetzel. In this manner the prospective potencies and values of a particular region

can be compared. In certain respects the results obtained by the two methods correspond, while in others they differ. For instance, Wetzel has found that the node region enters into the formation of the greater part of the embryo. Similarly, when such a region is isolated and grown as a graft, it has the capacity to differentiate most of the embryonic structures. On the other hand, certain regions which Wetzel has shown to form nervous system in the course of normal development do not have such a potentiality when isolated in a graft.

Waddington ('32) has employed a third method by growing parts of blastoderms *in vitro*. He has found the most active differentiation in those explants which contain the anterior part of the streak. He has also found it possible to induce a secondary medullary plate by grafting a part of the primitive streak between the germ layers of another blastoderm.

The present experiments are also closely related to the work of Willier and are, in fact, a part of a more comprehensive program undertaken by him and his students. The project was begun by transplanting the entire pellucid area of early blastoderms in different stages of development. The results of those experiments (Willier, '26; Willier and Rawles, '31 b) agree with the results obtained by transplanting isolated pieces in showing that the node is significant in the development of axial parts.

MATERIALS AND METHODS

The method of chorio-allantoic grafting has been so completely described by Willier and his students that it is not considered necessary to repeat it here. As usual the transplants were made to the chorio-allantoic membrane of chick embryo hosts of approximately 9 days' incubation. The incubation age of the donor blastoderms of a given stage varied considerably even from a single lot of eggs—a fact to be remembered by those working with very early stages. This variation cannot be avoided, as it is probably largely due to the fact that the eggs are collected only once or twice a day. The variations in specific instances may be as much as

5 or 6 hours and in warm weather it may be much more. Consequently, before the blastoderm was sectioned for transplanting, the stage of development was carefully noted and the primitive streak was measured. The length of the primitive streak is considered the most reliable criterion of the stage of development before the appearance of the head-process. Even so there are slight variations since the actual size of a given blastoderm does not always correspond exactly with its stage of differentiation.

RESULTS OF EXPERIMENTS

The grafts were obtained from blastoderms in which the head-process had not yet appeared—a stage that is designated as the definitive primitive streak. As may be seen in tables 1 a and 2, the length of the streak in this stage varied from 1.7 to 2.2 mm., with an average for eighty-two blastoderms of 1.93 mm. The incubation age varied from 12 to 21 hours, with an average of about 16 hours for the entire series. It is quite possible that in such a group there was a slight variation in the degree of differentiation of the blastoderm at the time of transplantation. The results, however, show a fair degree of uniformity, indicating that there are no great differences in the capacity for differentiation of the parts of the blastoderm in any one group. In a few cases a graft does not show as many structures differentiated as might be anticipated from the results obtained in other grafts from the same level. This is to be expected, since in some grafts there is incomplete incorporation and in others there is a more pronounced reaction of the host. Other factors also undoubtedly enter into the causes for the variation. It might also be noted that the table does not show the extent of growth of the various structures, but they are included in the table even though only a small part is identified. In some grafts there is a much more extensive growth of such structures than in others of the same level. No attempt has been made to analyze the factors causing these differences in growth rate, but undoubtedly there are many.

The position of the cuts that were made for the various transverse pieces may be ascertained by referring to figure 1, which is a diagram of a blastoderm in the definitive streak

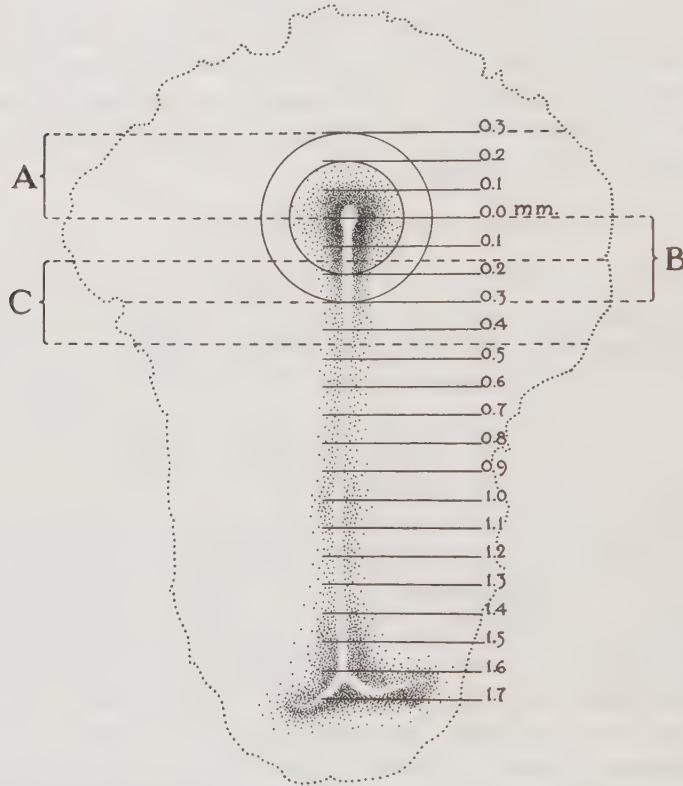


Fig. 1 Semidiagrammatic drawing of a definitive primitive streak stage embryo with the streak graduated into units of 0.1 mm. The inner and outer circles mark, respectively, the approximate positions of the boundaries of the more active center of development and of the node field. A photomicrograph of this embryo is shown in a previous report (p. 410, Hunt, '31 a).

stage. The streak, which has a length of 1.8 mm., is graduated into units of 0.1 mm. The center of the primitive pit is considered as 0.0 mm. since all cuts were made with reference to it.

Grafts of the level containing the anterior half of the node region

The grafts of this group were obtained from that part of the pellucid area which lies anterior to the primitive pit (level A, fig. 1). Two transverse cuts were made, one as nearly as possible through the center of the primitive pit and the other 0.3 to 0.4 mm. anterior to the first. The cuts extended laterally as far as the edge of the area pellucida and sometimes included a small part of the area opaca. Twenty-five grafts are included in table 1 a. Of the grafts obtained from this level only those which could be paired with grafts of the posterior half of the node are included. Thus in table 1 a and table 1 b the grafts of the same number (first column) are from the same blastoderm.

In general, some of the structures obtained in the grafts and their histological appearance are the same as those obtained in the grafts of the entire node level (compare table 3, level B, Hunt, '31 a). Consequently a complete histological description of some organs and tissues is omitted in order to avoid repetition.

Structures present in the grafts

The central nervous system shows a greater degree of growth and differentiation than in any other level transplanted. It is invariably present and in some cases is about the only structure definitely identified. Often, it is very extensive and may fill the greater part of the graft. Some regions can usually be further identified as prosencephalon and mesencephalon. Ventricular cavities are present in some cases, although they vary in size and are often irregular and abnormal in shape. Figure 2 is a photomicrograph which shows the degree of differentiation of the brain and eye. The blood in the ventricle of figure 2 is quite characteristic. In other cases the entire cavity is more or less filled with an extensive blood clot.

TABLE 1a AND 1b

Structures differentiated in the grafts from levels containing the anterior half and posterior half of the node

SERIAL NO. OF GRAFT	AGE OF DONOR	LENGTH OF STREAK IN MM.	NOTOCHORD	PROSENCEPHALON	MESENCEPHALON	NERVOUS TISSUE VENTR.	NERVOUS TISSUE WHORLS	NERVOUS TISSUE SOLID	GANGLION CELLS	EYE PIGMENT RET = D	RETINA = I, LENS = I	EPIPHYSIS	HYOPOPHYSIS	LUNG TISSUE	HEART	LIVER	THYROID	GUT WITH TUNICS	GUT WITHOUT TUNICS	PROVENTRICULUS	MESONEPHROS	ADRENAL	SKELTAL MUSCLE	CARTILAGE	MEMBRANE BONE	SKIN	FEATHER GERMS
1 103-42	14.00	1.9	+			+											+	+	+	+			+	+	+	+	+
2 104-6	12.00	1.8	+	+				+				+						+	+	+			+	+	+	+	+
3 110-6	14.20	2.3		+					p									+	+	+			+	+	+	+	+
4 110-34	16.25	2.0	+	+	+				pl	+							+	+	+	+			+	+	+	+	+
5 111-10	15.15	1.8	+		+			+					+		+	+		+	+	+			+	+	+	+	+
6 111-19	16.10	1.8	+			+							+					+	+	+			+	+	+	+	+
7 111-26	17.40	2.2	+	+				+	pr				+		+	+	+	+	+	+			+	+	+	+	+
8 112-17	17.00	1.9	+	+	+							+	+		+	+		+	+	+			+	+	+	+	+
9 112-21	18.00	2.1		+	+							+							+	+			+	+	+	+	+
10 112-24	17.50	2.0	+	+				+	pr			+						+	+	+			+	+	+	+	+
11 114-1	15.26	1.7		+	+			+	p								+	+	+	+			+	+	+	+	+
12 115-7	15.15	1.8	+	+	+			+	p									+	+	+			+	+	+	+	+
13 115-31	14.40	1.7	+	+				+	p			+						+	+	+	+	+	+	+	+	+	+
14 115-35	14.55	1.8		+	+			+	p				+	+	+		+	+	+	+			+	+	+	+	+
15 115-44	15.30	1.7	+		+			+					+	+			+	+	+	+	+	+	+	+	+	+	+
16 116-1	15.35	1.8	+	+				+	prl			+						+	+	+			+	+	+	+	+
17 116-28	17.30	1.8	+	+	+			+	p	+								+	+	+			+	+	+	+	+
18 117-1	16.30	1.9	+	+	+			+										+	+	+			+	+	+	+	+
19 120-10	16.35	2.1	+	+	+			+	prl	+	+	+	+	+	+			+	+	+			+	+	+	+	+
20 120-13	17.00	1.8	+	+				+									+	+	+	+			+	+	+	+	+
21 121-13	12.30	1.8		+					p									+	+	+			+	+	+	+	+
22 122-13	16.00	1.9	+	+				+										+	+	+	+		+	+	+	+	+
23 122-17	16.40	1.7	+		+			+										+	+	+			+	+	+	+	+
24 125-11	14.00	1.8			+			+										+	+	+			+	+	+	+	+
25 125-28	15.50	1.7	+	+				+										+	+	+			+	+	+	+	+
Totals			19	16	15	3			21	12	5	8	2	5	4	7		21	15	5	3		20	23	11	23	17
1 103-43						+		+					+	+	+			+	+	+			+	+	+	+	+
2 104-7								+	+									+	+	+			+	+	+	+	+
3 110-7			+		+			+					+	+	+			+	+	+			+	+	+	+	+
4 110-35			+		+	+		+					+	+				+	+	+	+	+	+	+	+	+	+
5 111-11			+		+			+					+	+	+			+	+	+			+	+	+	+	+
6 111-20			+		+	+		+					+	+	+			+	+	+			+	+	+	+	+
7 111-27						+	+	+					+	+	+			+	+	+	+		+	+	+	+	+
8 112-18			+		+			+					+	+	+			+	+	+	+		+	+	+	+	+
9 112-22						+	+	+					+	+				+	+	+			+	+	+	+	+
10 112-25						+	+	+					+	+				+	+	+	+	+	+	+	+	+	+
11 114-2						+												+	+	+			+	+	+	+	+
12 115-8								+					+	+				+	+	+			+	+	+	+	+
13 115-32													+	+				+	+	+			+	+	+	+	+
14 115-36			+		+	+		+					+	+	+			+	+	+	+	+	+	+	+	+	+
15 115-45			+		+			+					+	+				+	+	+			+	+	+	+	+
16 116-2						+							+	+				+	+	+			+	+	+	+	+
17 116-29						+	+	+					+	+				+	+	+			+	+	+	+	+
18 117-2								+					+	+				+	+	+			+	+	+	+	+
19 120-11			+		+			+					+	+				+	+	+	+	+	+	+	+	+	+
20 120-14			+		+	+		+					+	+				+	+	+			+	+	+	+	+
21 121-14			+		+		+	+					+	+				+	+	+	+	+	+	+	+	+	+
22 122-14						+	+	+					+	+				+	+	+	+	+	+	+	+	+	+
23 122-18					+	+		+					+	+				+	+	+			+	+	+	+	+
24 125-12			+		+		+	+					+	+	+			+	+	+	+	+	+	+	+	+	+
25 125-29						+	+	+					+	+				+	+	+	+	+	+	+	+	+	+
Totals			11		10	10	6	19					12	22	8			23	9	4	10	2	20	25	6	19	14

The rhombencephalon and spinal cord have not as yet been identified as such in the grafts of this level; nevertheless, it is possible that they are present. In three cases the region of the nervous system cannot be identified, but the histological appearance of the tissue is more like that of brain than spinal cord and, furthermore, brain-like ventricles are present.

In practically every case ganglion cells are present in the grafts, but in varying numbers in specific instances. They are found scattered in small or large groups.

Notochord is identified in 76 per cent of the grafts listed in table 1 a. The presence of the notochord and its correlation with the nervous system will be considered in greater detail in the discussion.

The development of the parts of the eye is essentially the same as in the grafts of the entire node. In two grafts the retina, pigmented layer, and lens are well differentiated but in most cases only the pigmented layer can be identified. Figure 2 shows a case in which there are well-developed retinal layers and three distinct lenses.

Of the other ectodermal structures, epiphysis appears in five grafts and hypophysis in eight. Of the latter only the pars buccalis has been identified. In some grafts a connection can be traced between it and the oral cavity. Skin is often present, and in seventeen of the cases there are well-developed feather germs.

Gut as well as nervous system is present in all of the grafts. In some cases a specific part of the gut is seen. The proventriculus, for instance, is identified by the rounded gland primordia present in the mucosa. Other specific parts of the digestive tube, such as the pharynx and duodenum, are undoubtedly present also, inasmuch as thyroid and liver are found. No special study of these divisions has been made, however, and it is not always possible to identify them from their histological appearance alone. In most cases the gut is present merely as a longitudinally-folded, epithelial tube around which a connective tissue and a circular muscle layer have usually differentiated. The gut occurring in the grafts

does not necessarily form a continuous tube and there may be several unconnected parts of it present.

Thyroid is identified in seven and respiratory tissue in two of the grafts. The former is indicated by small follicles containing an eosin-staining, colloid-like substance. These follicles are mingled with numerous bud-like masses of cells arranged in anastomosing cords. The bronchial tubes of the lung are seen as epithelial-lined cavities which are surrounded by small knobs or plates of cartilage and condensed mesenchyma. In some cases the plates are still in the pre-cartilage state. Liver, which has been fully described elsewhere (Hunt, '31 a, Willier and Rawles, '31 a), is present in only four of the twenty-five grafts of this level.

Correlated with the appearance of liver is the presence of heart tissue. Willier and Rawles ('31 a) have made an extensive examination of this correlation, and the present writer ('31 a) confirmed their observations. The cases cited here may be considered as additional evidence of the relation between these two structures.

Mesonephros is also found to develop in three of the grafts. While it occurs less frequently and is usually not so abundant as in the grafts of the posterior half of the node, the few tubules that do form are characteristic and have capsule, secretory, and collecting parts.

Of the other mesodermal structures, cartilage, muscle, and membrane bone are found. The cartilage is usually scattered in irregular masses of varying size. The skeletal muscle is often abundant and may or may not be found attached to the cartilages by pre-tendon tissue. Perichondrial bone has not yet appeared, probably because the grafts have not grown for a sufficiently long time.

Grafts of the level containing the posterior half of the node

These grafts were obtained from the same blastoderms as those of the previous group. The parts taken for transplantation included a transverse strip, 0.3 to 0.45 mm. in antero-posterior width (level B, fig. 1), the anterior cut of the piece

passing through the center of the primitive pit in each case. Such a piece included the posterior half of the node and the area pellucida lateral to it. Twenty-five grafts were obtained of this region which could be paired with grafts of the anterior half of the node. In table 1 b are listed the structures found in the grafts.

Structures present in the grafts

Nervous tissue predominates in these grafts just as it did in the grafts of the level including the anterior half of the node. However, the character of it is very different. Instead of being present as fairly well-formed brain or neural cord, it is usually unorganized, and it is difficult and often impossible to identify it as belonging to any specific part of the central nervous system. In six grafts it is seen as an irregular mass of tissue like that shown in figure 5. In nine of the grafts, where notochord also occurs, there is a ventricle-like cavity with a thin roof which might represent hindbrain, although it cannot be definitely identified as such. Figure 3 shows an example of this type of differentiation. There are also ten cases in which there are small areas of nervous tissue that contain one or more small lumina. It is not considered that such lumina represent the central canal of the neural tube, but rather that they are whorl formations of ependymal cells, such as Maximow ('25) described in his cultures of rabbit embryos. An example of such is shown in figure 4.

Ganglion cells are quite as numerous as in the grafts of the more anterior level, and they have essentially the same structure.

Notochord occurs less frequently, being present in only eleven of the twenty-five cases, or in 44 per cent of the grafts listed. When it does occur, it is usually as extensive in amount as in the grafts of the more anterior level.

Of the other ectodermal structures, skin and feather germs are the only ones represented. Both are present in the majority of grafts.

Gut is present in every case. As in the grafts of the more anterior level, the proventriculus is found occasionally. The occurrence of respiratory tissue and liver, which are each present in twelve and eight cases, respectively, shows that other specific parts of the gut are also represented. In all cases in which liver is present, it is found in association with heart tissue.

Heart is found in all but three of the grafts. It is to be noted that it is present in the five grafts which are mates of the grafts of the more anterior level in which heart tissue is also found. No difference is noted in the form or histological appearance of the heart tissue found in the grafts of the posterior half of the node and that found in the grafts of other levels.

Mesonephros is found in ten of the twenty-five grafts of this level, which is a much greater frequency than occurred in the cases of the more anterior level. Furthermore, a greater number of tubules is usually present in individual grafts. Adrenal gland is associated with the mesonephros in two grafts, whereas in the anterior level it has not yet been found to occur.

The frequency of occurrence and the histological appearance of cartilage and skeletal muscle are essentially the same as in the grafts of the more anterior level. Membrane bone, while having the same structure, appears somewhat less frequently.

Grafts of the level containing the posterior part of the node region

The grafts of this group (table 2) were obtained from a level of the primitive streak which, although posterior to the primitive pit, contained a part of the node region. Level C, figure 1, shows the piece usually taken for transplantation, but under this heading strips are included in which the anterior level of transection varies from 0.1 to 0.3 mm. posterior to the center of the pit. The antero-posterior width of the strip varied from 0.3 to 0.45 mm. It is to be noted that the



Structures differentiated in the grafts from levels of the streak posterior to the primitive pit

SERIAL NO. OF GRAFT	AGE OF DONOR	LENGTH OF STREAK IN MM.	M.M. POSTERIOR TO PIT	NERVOUS TISSUE WHORLS	NERVOUS TISSUE SOLID	GANGLION CELLS	HEART	MESONEPHROS	ADRENAL	GUT WITH TUNICS	GUT WITHOUT TUNICS	SKELETAL MUSCLE	CARTILAGE	MEMBRANE BONE	SKIN	FEATHER GERMS	EPIDERMIS	SERIAL NO. OF GRAFT	AGE OF DONOR	LENGTH OF STREAK IN MM.	M.M. POSTERIOR TO PIT	GUT WITH TUNICS	GUT WITHOUT TUNICS	SKELETAL MUSCLE	CARTILAGE	SKIN	FEATHER GERMS	EPIDERMIS
(a106-17)	17.30	2.2	0.1	+						+	+	+	+	+	+	+		(c108-15)	17.00	2.0	0.47	+	+	+	+	+	+	
106-45	21.00	2.1	0.1		+	+	+	+	+	+	+	+	+	+	+	+		109-4	12.00	2.2	0.47	+			+	+	+	
129-11	16.00	2.1	0.1		+	+	+	+		+	+	+	+					135-12	13.20	1.7	0.51	+			+	+	+	
(b152-17)	15.00	1.7	0.1	+		+				+	+	+	+		+			(f135-22)	13.55	2.2	0.51	+				+	+	
(c108-14)	17.00	2.0	0.15												+	+		152-38	15.15	1.7	0.51	+				+	+	
126-27	17.15	2.2	0.15	+	+	+		+	+	+	+	+	+		+	+		(k131-5)	12.0	1.9	0.54	+				+	+	
129-23	16.45	1.8	0.15							+	+	+	+		+	+		Totals			12				6	8	7	
129-31	17.30	2.1	0.15		+	+				+	+	+	+		+	+		(g115-3)	15.15	1.7	0.6	+	+			+	+	
130-23	17.35	1.8	0.15		+	+	+			+	+	+	+		+	+		118-17	16.45	2.1	0.6	+			+	+	+	
(d135-15)	13.30	2.0	0.15		+					+		+	+		+	+		(e120-33)	17.50	2.1	0.6	+			+	+		
106-37	20.15	1.9	0.15							+	+	+	+					127-39	20.15	1.8	0.6	+						
109-15	12.30	1.9	0.15	+		+		+		+	+	+	+	+	+	+		(h152-20)	14.35	2.1	0.6	+						
132-12	17.20	2.0	0.15		+	+		+	+	+	+	+	+		+	+		112-14	17.21	2.0	0.65	+						
107-25	16.45	2.0	0.18							+	+	+	+					(i112-28)	18.10	2.0	0.65	+			+	+	+	
110-24	15.45	2.0	0.18	+	+	+	+			+	+	+	+		+	+		(j112-33)	18.20	2.2	0.75	+			+	+	+	
119-7	16.20	2.1	0.18		+	+	+			+	+	+	+		+	+		130-12	16.45	2.1	0.75	+				+	+	
(e120-32)	17.50	2.1	0.2							+	+	+	+		+	+		(i134-34)	16.35	1.9	0.75	+			+			
132-20	18.00	2.1	0.2		+	+				+	+	+	+		+	+		113-18	16.05	2.2	0.77	+						
134-8	15.05	2.0	0.2															Totals			11	1			6	6	5	
(f135-21)	13.55	2.2	0.2	+	+	+				+	+	+	+		+	+		107-8	17.00	1.7	0.8	+					+	
152-13	14.20	1.8	0.2		+	+	+			+	+	+	+		+	+		108-36	18.20	2.2	0.8	+			+			
(g115-2)	15.15	1.7	0.2		+					+	+	+	+		+	+		152-57	16.10	1.8	0.9	+				+		
125-35	15.05	1.8	0.21					+		+	+	+	+					134-49	20.10	2.2	0.96	+						
126-17	16.50	1.8	0.24		+	+				+	+	+	+		+	+		(d135-18)	13.30	2.0	1.05	+	+					
132-16	17.45	1.8	0.24		+	+		+	+	+	+	+	+		+	+		(a106-20)	17.30	2.2	1.11	+				+		
134-23	15.55	1.8	0.24			+		+		+	+	+	+			+		106-29	19.00	1.9	1.11	+						
(h152-19)	14.35	2.1	0.24		+					+	+	+	+		+			109-12	12.15	2.0	1.11	+						
(i134-33)	16.35	1.9	0.27			+				+	+	+	+		+	+		(k131-7)	12.00	1.9	1.2	+	+					
(j112-32)	18.20	2.2	0.28		+	+	+			+	+	+	+		+	+		(i134-35)	16.35	1.9	1.2	+	+				+	
107-3	15.45	1.7	0.3			+				+	+	+	+		+	+		(l112-30)	18.10	2.0	1.25	+						
129-19	20.10	2.2	0.3							+	+	+	+		+	+		152-46	15.45	1.8	1.35	+	+			+		
Totals				5	13	21	12	13	3	27	9	20	28	4	27	23	1	144-40	15.25	1.9	1.45	+				+		
129-39	17.30	1.8	0.36							+		+			+	+		142-29	16.45	2.2	1.5	+						
152-39	16.00	1.8	0.36							+					+			142-6	15.30	2.1	1.53	+					+	
107-42	18.40	2.0	0.38									+						148-18	14.35	2.0	1.55	+						
(b152-18)	15.00	1.7	0.4							+	+							Totals			12	4				5	3	
(d135-16)	13.30	2.0	0.4							+		+			+	+												
108-4	16.15	1.8	0.47							+					+	+												

measurements are only approximately accurate. Measurements were taken before the cuts were actually made, and there is a possibility of error in cutting of as much as 0.05 mm., even after considerable experience. It is partly because of this possible error that these grafts and those to be described below are treated as groups. It is also to be noted that grafts of this series overlap part of the region which was included in the level containing the posterior half of the node. This overlapping of the level transplanted should be remembered in considering the development and the relative frequency of occurrence of certain structures and in making comparisons between these grafts and those just described.

Structures present in the grafts

Central nervous system is found in eighteen of the thirty-one grafts of the levels which are between 0.1 and 0.3 mm. posterior to the pit. It is not the predominant tissue as is the case in the grafts of the more anterior levels, but is present only in small amounts. Furthermore, it is not organized as to form; i.e., a neural tube is not formed in any case nor has a specific part differentiated. Figure 5 shows the character of the nervous tissue which has differentiated in most cases. No central canal or whorls are present in these grafts. In five of the grafts, from levels between 0.1 and 0.15 mm. from the pit, there is an ependymal whorl formation similar to that mentioned above and shown in figure 4.

On the other hand, the peripheral nervous system in the form of ganglia and nerves, is as well differentiated as in the grafts of more anterior levels. In five cases, from levels 0.2 mm. or more posterior to the pit, ganglia and nerves are the only parts of the nervous system represented. Occasionally smaller ganglion cells, presumably of the sympathetic system, are also found. It is to be noted that notochord is not present in any of the grafts of this group.

Of the entodermal structures gut is the only one that is definitely present and it occurs in all grafts. As yet, no

specific part of it has been identified with certainty, and it is usually seen merely as an epithelial tube surrounded in all but four cases by tunics of mucosa and smooth muscle.

Heart tissue similar to that found in the grafts of more anterior levels is identified with certainty in twelve grafts. Mesonephros occurs in thirteen grafts, and in three of these adrenal gland is associated with it.

Cartilage is present in most cases, and the frequency of occurrence of skeletal muscle is essentially the same as in grafts of more anterior levels. Membrane bone occurs in only four of the grafts. Skin and feather germs appear with somewhat greater frequency in the grafts of this level, but it is not certain that the difference is sufficient to be significant. Possibly, this indicates that this level has a greater potentiality to produce trunk structures since feather germs are formed earlier in the trunk than in the head region.

Grafts of streak levels posterior to the node region

Altogether thirty-nine grafts have been examined which were from levels of the primitive streak at varying distances posterior to the primitive pit and node region. Only rarely was it possible to obtain grafts from all levels of a single blastoderm. Cases in which more than one graft was obtained from a blastoderm are shown in the first column of table 2 by parentheses. Greater difficulty was experienced in obtaining grafts from the posterior levels of the primitive streak than from the more anterior. In many cases there would be merely a slight reaction of the host membrane and no differentiation in the graft.

The grafts are more or less arbitrarily grouped for descriptive purposes. The first group includes grafts from levels 0.36 to 0.54 mm. from the primitive pit, the second from 0.6 to 0.77 mm., and the third from 0.8 to 1.55 mm. The distance from the center of the pit to the anterior transecting cut of each strip transplanted is shown in the fourth column of table 2. The strips extended laterally to the area opaca and the antero-posterior width varied from 0.3 to 0.45 mm.

a. *From 0.36 to 0.54 mm. posterior to the pit.* In the twelve grafts of this group no nervous tissue or any other structures which might be considered specific as to the level of the axis can be identified. The only structures appearing with a frequency approximately equal to that of the grafts of more anterior levels are non-specific gut, skin, and feather germs, all of which are similar to those found in the grafts containing parts of the node region. There is a significant decrease from 90 per cent to 50 per cent in the frequency of occurrence of cartilage, and the relative amount is also greatly diminished. Except for the doubtful presence of skeletal muscle in two cases, no other structures have been identified in these grafts.

b. *From 0.6 to 0.77 mm. posterior to the pit.* There are no significant differences between the eleven grafts of this group and those just described. The frequency of occurrence and histological appearance of gut, cartilage, skin, and feather germs are essentially the same.

c. *From 0.8 to 1.55 mm. posterior to the pit.* In this group of sixteen grafts there is a decrease in capacity to form even those structures which are non-specific as to the level of the axis. There is also somewhat greater difficulty in obtaining grafts from these levels than from those more anterior in position. Gut occurs in all cases in which there is any differentiation and is usually similar in structural appearance to that already described. It is usually not so abundant, however, and in four cases no muscular tunic is developed. Neither cartilage nor feather germs appear in any of these grafts. Small quantities of skin occur in five cases, and in three others there are areas of slightly stratified epithelium which may possibly be epidermis. The underlying mesenchyma, however, is not sufficiently vascularized or condensed to be considered as dermis.

DISCUSSION

In considering the grafts from the various transverse levels of the area pellucida, it is apparent that there is a gradient in developmental potencies. These potencies are greatest in the level containing the anterior half of the node and gradually decrease as more posterior levels of the node are approached. The primitive streak behind the node shows first a marked and then a gradual diminution of capacity. The region anterior to the node level of the primitive streak was shown in earlier experiments (Hunt, '31 a) to have very little capacity, if any, for differentiation. The gradient is evidenced: 1) by the kinds of structures and tissues which develop in the grafts of different levels; 2) by the more frequent occurrence in a particular level of structures common to several levels; 3) by the greater degree of differentiation of some structures in the grafts of certain levels than in others.

Structures characteristic of different levels of the axis. The level containing the anterior half of the node, i.e., the part lying anterior to the primitive pit, has the potency to differentiate the greatest variety of structures. Certain organs, including eye, epiphysis, hypophysis, and thyroid, develop in grafts of this level only. Others, such as nervous system, notochord, heart, liver, lung, mesonephros, and membrane bone, are found not only in grafts of this level, but also in the grafts of the levels containing the posterior half of the node, i.e., the part lying just posterior to the primitive pit. In addition, the grafts containing the posterior half of the node form adrenal gland, but the total number of kinds of structures is less than that in grafts of the more anterior level. There is likewise a slight diminution in capacity for differentiation of various kinds of structures in the grafts of levels containing the more posterior region of the node, i.e., levels between 0.1 and 0.3 mm. posterior to the primitive pit. Notochord does not appear nor has liver yet been found to develop in grafts of this last group.

All of the grafts mentioned thus far have developed from levels of the node region. In levels below this region there is a very noticeable diminution in developmental potencies. In contrast to the great variety of tissues and organs formed in the grafts of the more anterior levels gut, cartilage, skin with feather germs, and possibly skeletal muscle are the only structures formed in the levels 0.3 to 0.54 mm. posterior to the pit. All of these structures develop in grafts of the node levels as well. In levels between 0.6 and 0.77 mm. there is no decrease in capacity to produce kinds of structures. Posterior to 0.8 mm., however, there is a further decrease in the variety of structures formed, since cartilage and feather germs do not develop.

Frequency of occurrence of structures. It is also noted that there is a difference in the frequency with which these structures appear along the antero-posterior axis. Notochord, for instance, develops in 76 per cent of the grafts of the level containing the anterior half of the node, whereas it appears in only 44 per cent of those containing the posterior half. In the case of heart, the frequency is greatest in the grafts of the level containing the posterior half of the node where it occurs in 88 per cent of the cases. This may be contrasted with its occurrence of 20 per cent in the grafts of a more anterior level and 39 per cent in those of the level containing the more posterior part of the node region. Membrane bone appears in 44 per cent, 24 per cent, and 13 per cent of the grafts of levels which include, respectively, the anterior half, the posterior half, and the more posterior region of the node.

No difference in the occurrence of cartilage, skeletal muscle, skin, and feather germs is apparent in the levels containing a part of the node, but there is a great difference in the capacity to produce these structures in the levels behind the node region. For example, cartilage is almost invariably present in all of the grafts of the node levels. In the twenty-three grafts of levels between 0.3 and 0.77 mm. posterior to the pit, however, the frequency falls to 48 per cent, and in the grafts of levels 0.8 mm. or more posterior it is entirely

absent. Likewise, skeletal muscle occurs in the majority of the grafts of node levels, but it is not identified with certainty in any of the grafts of levels more than 0.3 mm. posterior to the pit. In the case of skin and feather germs there is no marked difference in occurrence in grafts of levels as far back as 0.77 mm. Beyond this level, however, feather germs have not been found to develop and skin occurs in only five of the sixteen grafts which have been examined. There may also possibly be a decrease in frequency of gut in the grafts which include only the posterior end of the streak. This is not apparent in the table since many of the grafts showed no differentiation and consequently were not included.

The degree of differentiation of structures. It is further seen that there is a gradient in the degree of differentiation and growth of certain structures. The degree of differentiation is indicated by the quality of form and by the stage reached in histogenesis; the growth, by the relative amount of tissue formed. Such qualitative and quantitative differences are best seen in the development of the central nervous system. In the grafts of a level which contains the anterior half of the node well-formed brain appears in practically every case. Brain vesicles are present which can usually be identified by the structure of their walls as fore- and mid-brain. Moreover, the quantity of the brain tissue is greater than in any other level. In the next level posterior, i.e., one which includes the part of the node lying just back of the primitive pit, the central nervous tissue, while often rather abundant, is less well-developed than that of the more anterior level. In no graft can it be identified as belonging to a specific region of the central nervous system, although in some instances where a ventricle-like formation with a thin roof is present, the tissue does resemble, to a certain extent, the hind-brain in form (fig. 3). In other cases no ventricular cavity or central canal is present and the tissue appears either in the form of ependymal whorls, as in figure 4, or as solid masses, as in figure 5. In the grafts of levels 0.1 to 0.3 mm. posterior to the pit which contain the more posterior part

of the node region, there is a still further decrease in capacity for the differentiation of nervous tissue. Specific central nervous tissue develops in eighteen of the thirty-one grafts, but is less abundant and less well-organized as to form, i.e., a neural tube is not formed in any case. No central nervous tissue appears in any of the grafts of levels more than 0.2 mm. posterior to the pit. It is to be noted that while all levels of the node possess the power to differentiate central nervous system, there is a marked difference in the degree of specialization attained. Peripheral nervous system in the form of ganglia and nerves, on the other hand, develops equally well in all levels which include the node region in the transplant.

In the case of gut, there are also variations in the degree of differentiation in the grafts of the various levels. Although it occurs in some form in practically all grafts, specific parts develop only from levels which contain a part of the node. The proventricular region, for instance, may be identified in such grafts as the latter by the gland primordia. Other specific regions, such as the pharynx and duodenum, are undoubtedly present, since thyroid and liver develop in the grafts. Since no special study of the histological structure of the digestive tube present in the grafts has yet been made, all parts have not been identified with certainty. In all levels posterior to the node region the gut appears merely as an epithelial tube usually surrounded by tunics of connective tissue, circular muscle, and occasionally longitudinal muscle.

Skin also shows some variation in the degree of differentiation which occurs. Its development is essentially the same in grafts of all levels of the node region and of the primitive streak as far posterior as 0.77 mm. behind the pit. In levels more posterior the skin, if present at all, is less well-developed. Feather germs are absent and only small areas containing both dermis and epidermis are present. More often epidermis which is only slightly stratified occurs without an underlying corium.

The question of localization. The preceding evidence shows that there are qualitative differences in the developmental

potencies of the various transverse strips of the axis. Certain structures appear with a greater frequency or are further differentiated in grafts from a particular level than from any other. What is the significance, then, of these facts in relation to the general problem of localization? It appears that two possible interpretations may be considered. One, that segregation has occurred prior to the time of transplantation, i.e., that the blastoderm is a mosaic of specific potencies which become realized by self-differentiation in the graft. The other is that at least some of the specific potencies arise by an epigenetic process. The latter point of view is one developed by Willier (unpublished) from his chorio-allantoic grafting experiments.

If structures are segregated, then it must be assumed that the segregates were split when transections of the area pellucida were made for transplantation. Such a splitting might have occurred in the case of heart, for instance, since it is found to develop in two levels of the same donor which contain, respectively, the anterior and posterior one-half of the node. Heart also appears in grafts of levels as much as 0.3 mm. posterior to the primitive pit. It thus appears that the heart-forming area is a relatively large field. There is a region of this field, however, viz., the level containing the posterior half of the node, which has a much greater capacity to produce heart than any other. In grafts of levels anterior and posterior to this region the potency to produce it is much less, as shown by the lowered frequency of its occurrence. It is possible, however, that the heart normally develops only from the level containing the posterior half of the node and that its development in other levels is epigenetic. Thus the grafts do not necessarily show that specific potencies of the structures which differentiate are localized or segregated at the time of transplantation nor do they indicate that such structures have arisen by self-differentiation. They merely demonstrate the potentiality for differentiation of an isolated piece that includes three germ layers. At the present time we are attempting to answer more definitely this question, of

whether or not structures are localized and are self-differentiating, by transplanting parts of a single germ layer which would thus be removed from any influence of the other germ layers.

Whereas the evidence that the blastoderm is a mosaic of specific potencies is inconclusive, there is definite proof that epigenetic development does take place in the grafts. The work of Willier and the present experiments demonstrate that this is true. For instance, in the development of the eye, it is occasionally found that more than the lens is formed. Figure 2 shows such a case in which three lenses occurred instead of one. It is doubtful whether there were three segregates in the level from which this graft was obtained. As further evidence, the experiments of Wetzel ('29) in relation to these experiments may be cited. He has shown by vital staining that an area of the primitive streak posterior to the node region has the prospective value of nervous tissue (Abb. 111, p. 301, '29). This area, however, when isolated and grown in a graft lacks the potency to differentiate nervous tissue. Thus there are cells which produce nervous tissue in normal development, but which are still undetermined and do not have the capacity for self-differentiation when isolated in a grafted piece. This raises the question of the process whereby nervous system is differentiated and the possible rôle of the node in its determination.

The relation of the node to the determination of the central nervous system. The relationship between the development of the head-process and nervous system has been discussed previously (Hunt, '31 a). At that time it was suggested that the differentiation of the neural plate was brought about by the inducing capacity of the head-process, or more specifically, the chord-anlage. The present experiments tend to support this conclusion, since there is a striking parallel between the degree of differentiation of the nervous system in the grafts of the levels that contained a part of the node and the frequency of occurrence of notochord in those same levels. In the grafts that include the anterior half of the node, the

notochord is present in 76 per cent of the cases, the same percentage of frequency that occurred when the entire node region was transplanted in the earlier experiments. It is in this group that the nervous system shows the most normal development of form, quantity and specific histological structure. In the grafts of the level that included the posterior half of the node the notochord occurs in only 44 per cent of the grafts. Not only is the frequency of occurrence of notochord decreased in these grafts, but the nervous system is less well-developed, especially in fourteen of the twenty-five cases in which notochord is absent. In nine of the eleven grafts in which notochord does appear, the nervous tissue is better organized at least in form. The notochord is invariably absent in the grafts in which the more posterior region of the node was included. In thirteen of these grafts central nervous tissue does not occur, and in the other eighteen cases there are only small amounts which are unorganized as to form and non-specific as to the level of the neural tube. In general, it may be said that the nervous system developed most normally in the grafts where notochord is most frequently present. Conversely, in the grafts where the nervous system is most poorly developed and most unorganized, the frequency of notochord is greatly diminished or this tissue is absent altogether.

In individual grafts this is usually the case, but it does not always hold true. In a few instances in the grafts of levels containing the anterior half of the node (grafts 112-21, 114-1) or the posterior half (122-18) the notochord is not present and the nervous tissue is as well-developed as in the cases in which the notochord is present. This may possibly be explained, however, by the fact that the chord-anlage does not always develop into notochord. There is also a possibility that even though the notochord started to develop, it was absorbed during the development of the graft. Grafts of the level anterior to the node of the head-process stage (Hunt, '31 a) show that some such explanation is plausible, since in many of those grafts notochord did not develop, although a definite

primordium had been established at the time of transplantation.

While it thus seems probable that there is some interrelationship in the development of nervous system and head-process, the facts also suggest that the nervous system is not completely dependent upon the head-process for its development. In the grafts containing the more posterior part of the node region there is some development of nervous system. Although the capacity for its differentiation is very limited, the fact remains that it did develop in the absence of notochord. There is a possibility that the cells which formed this nervous tissue may have been determined before transplantation because of their proximity to prospective chorda. Assuming, however, that the results do indicate at least a partial independence in the development of nervous tissue, what rôle may the head-process have in such development? The preceding evidence suggests the conclusion that cells which may have been determined before transplantation form tissue characteristic of a specific level of the central nervous system only when acted upon by the chord-anlage. Furthermore, the chord-anlage may be essential to the morphogenesis of the neural tube.

The limits of the node and node field

It is noted from the preceding evidence that a marked differentiation of structures will occur only when a part of the node or the region immediately surrounding it is included in the transplanted part. The relatively large number of grafts obtained from streak levels posterior to the node region (0.3 mm. or more behind the pit) shows conclusively that this is the case in this type of experiment. In this connection it is interesting to note that Waddington ('32), using a different method, has obtained not only a differentiation of medullary plate from the middle third of the primitive streak, but also an induction of a secondary medullary plate by this same region. Possibly this apparent discrepancy in results may be explained by a misunderstanding of terms such as 'middle

one-fifth' or 'one-third' in designating a part of the streak. Moreover, since the growth rate of Waddington's donor blastoderms was apparently retarded, it seems quite probable that the length of the streaks was also diminished. If this were the case, then a middle one-third of the streak would include a region relatively nearer the primitive pit. Consequently, until a more specific knowledge is forthcoming of the streak lengths in individual cases and the distance posterior to the pit of the middle one-third of the streak from which medullary plate is obtained, it will not be possible to make a fair comparison between Waddington's results and those obtained by chorio-allantoic grafting.

Judging from Waddington's report as well as others, there is a difference of opinion as to just what is meant by the node. In my report the node is considered to be the accumulation of cells which surround the primitive pit anteriorly and laterally and merge imperceptibly with the primitive folds posteriorly. As seen in figure 1 of the definitive primitive streak stage, it appears as the much darker area surrounding the pit. The node is thus a slightly elongated, circular or disk-shaped area between 0.3 and 0.4 mm. in diameter and is indicated in figure 1 by the stippled area within the smaller inner circle. It is this region bounded by the inner circle which has the greatest capacity for differentiation and which may be considered as the center of development.

Surrounding this center of greater potentiality is a field which has a lesser capacity for differentiation, the capacity diminishing as the periphery of the field is approached. The periphery of this field in blastoderms of the definitive primitive streak stage is between 0.25 mm. and 0.3 mm. from the center of the primitive pit and is shown in figure 1 by the outer circle. It will be seen in table 2 that the only structures which develop in grafts more than 0.3 mm. posterior to the pit are gut with tunics, muscle, cartilage, skin, and feather germs. Grafts obtained from a region approximately the same distance anterior to the pit show no greater differentiation. This is shown by the results of the earlier experiments

(Hunt, '31 a, p. 413). Grafts have also been obtained from regions lateral to the primitive pit, and in those which were more than 0.3 mm. lateral, there is very little differentiation. A more complete discussion of the results obtained by transplanting the regions lateral to the primitive streak will be made in another report.

The larger field of development mentioned above, which is approximately 0.6 mm. in diameter, is somewhat greater than Hensen's node is usually considered to be. The node was measured in the living condition from the surface and as is known from viewing sections of blastoderms through the node of this stage, it is not so sharply delimited from the surrounding parts as a surface inspection would seem to indicate. There is a gradual transition zone which marks the periphery, and it is this zone which also marks the periphery of the above mentioned field. In order to avoid a confusion of terms, it is proposed to designate this larger area as the 'node field' to distinguish it from the node proper which is the more active region of development. The node field corresponds more or less to the 'Scheibe' of Wetzel, but it is considered that the former term is more appropriate, since it more adequately describes a region of development of which the node is the center.

SUMMARY

Chorio-allantoic grafts from various transverse levels of the area pellucida, which include parts of the node and primitive streak, show that there is a gradient in developmental potencies. These potencies are greatest in the level containing the anterior half of the node and gradually decrease as more posterior levels of the node region are approached. Beyond this region there is first a marked and then a gradual diminution of capacity in the more posterior levels of the streak. The gradient is evidenced by the kinds of structures and tissues which develop in the grafts of different levels, by the more frequent occurrence in a particular level of structures common to several levels, and by the greater degree of differentiation of some structures in the grafts of certain levels than in others.

In general, the nervous system develops most normally in the grafts containing the anterior part of the node where notochord develops most frequently. Conversely, in the grafts containing the more posterior part of the node region, the nervous system is unorganized and poorly developed and the notochord is invariably absent. The results thus give further evidence of the existence of a developmental correlation between nervous system and notochord.

Judging from the results of the experiments, the center of development is considered to be a circular area 0.3 to 0.4 mm. in diameter. This corresponds approximately to the position of Hensen's node. Surrounding this center of greater potentiality is an area 0.5 to 0.6 mm. in diameter which has a lesser capacity for differentiation and which has been designated the 'node field.'

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PLATE 1

EXPLANATION OF FIGURES

2 Photomicrograph of a section from graft 116-1, level A, showing brain wall of the prosencephalon (*p*), the pigmented (*pr*) and sensory (*re*) layers of the retina, three lenses(*l*), and cartilage (*c*). $\times 39$.

3 Photomicrograph of a section of graft 125-12, level B, showing nervous tissue (*nt*) more poorly developed than that in figure 2, but having a ventricular cavity with a thin roof (*r*). There is also a whorl formation of ependymal cells (*w*), a small notochord (*n*), and a mass of cartilage (*c*). $\times 84$.

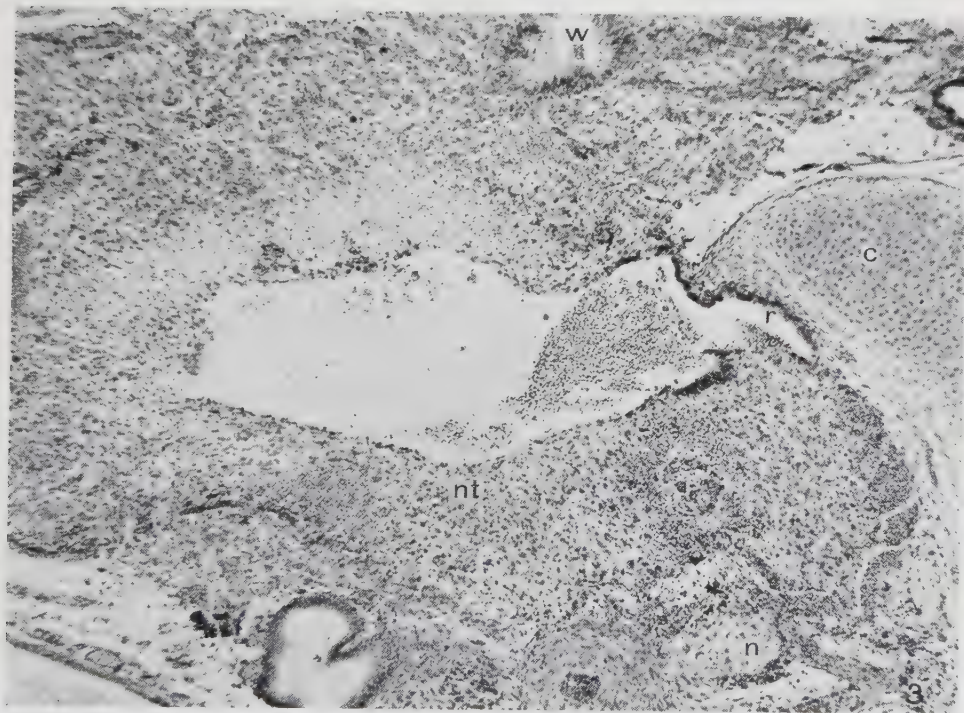
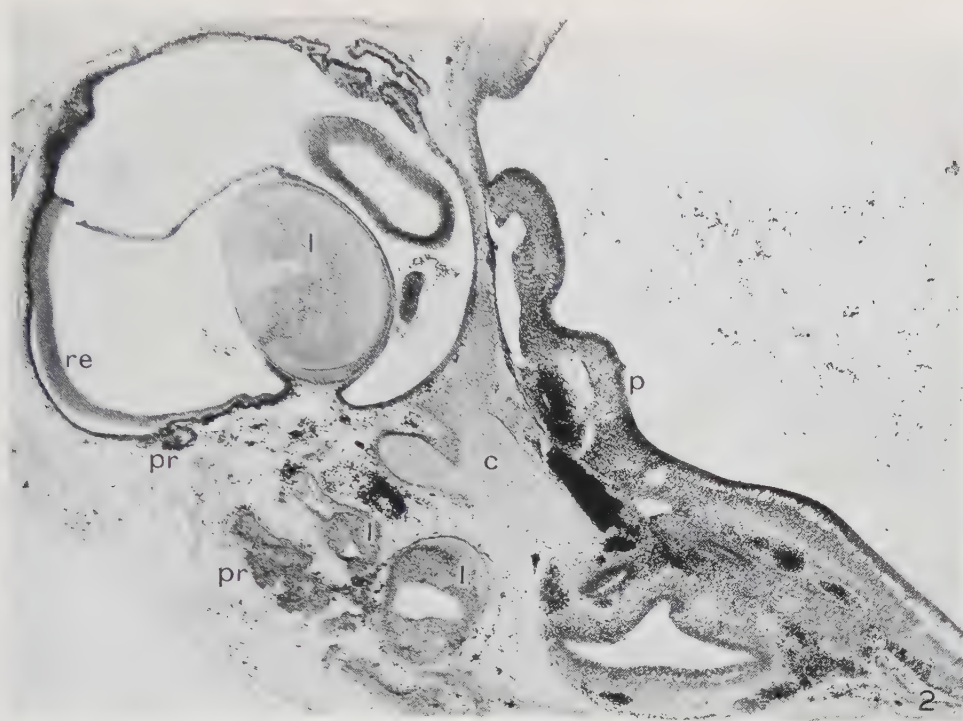
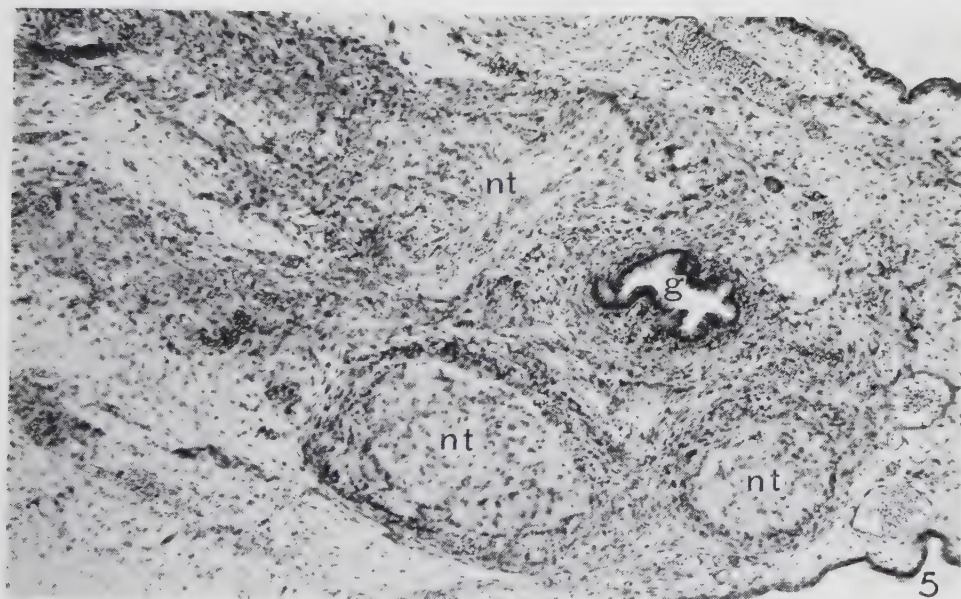
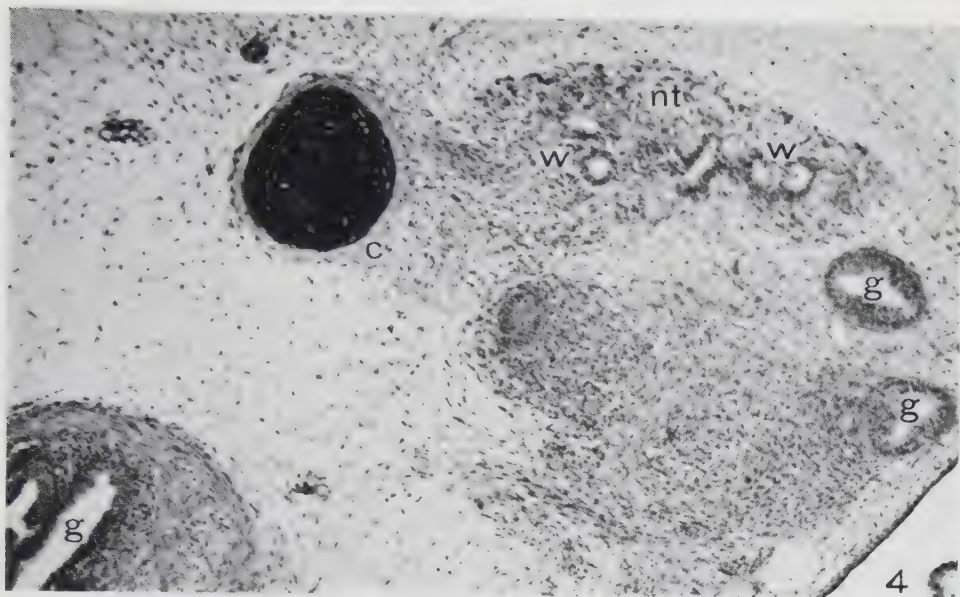


PLATE 2

EXPLANATION OF FIGURES

4 Photomicrograph of a section of graft 116-29, level B, showing a whorl formation of ependymal cells (*w*) in the midst of a small mass of central nervous tissue (*nt*). There is also shown gut with tunics of connective tissue and circular muscle at the left (*g*), and two small gut tubes without tunics at the right. $\times 105$.

5 Photomicrograph of a section of graft 119-7, level C, showing three small, solid masses of central nervous tissue (*nt*) and also gut without tunics. $\times 105$.



THE PROBLEM OF THE DOUBLE DUCTUS CHOLEDOCHUS (AN INTERPRETATION OF AN ACCESSORY BILE DUCT FOUND ATTACHED TO THE PARS SUPERIOR OF THE DUODENUM)

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FIFTEEN TEXT FIGURES AND ONE PLATE

The rare occurrence of two common-bile ducts in man seems to have attracted little attention from contemporary students of pathologic anatomy, judging from the latest comprehensive résumés of the biliary duct system (Anders, '28; Hanser, '29 and '30). Perhaps this is due to the all but recent absence of attested modern cases. Yet it is not without significance that the earliest systematic dissection of the human body was ushered in by a controversy over the insertion of the ductus choledochus; and that, three centuries later, the first embryologic studies of the origin of the liver raised a similar issue, namely, as to whether there were not normally two hepatic outgrowths from the duodenum.^{1, 2}

The dissecting-room case which is herewith presented³ is believed to be of significance in that it raises anew the question as to how much of the foregut retains its potency for forming hepatic tissue, and because it provides

¹ In the year 1500, Gabriel de Zerbis maintained, and it was asserted as late as 1605 by Graseceius, that two ducts always sprang from the 'Chistis fellea,' of which one emptied into the duodenum and the other into the fundus of the stomach. Vesalius, however, denied that this was the regular arrangement, for he had seen it only once (1543); so, also, it was denied by Fallopius (1606), who mentioned, however, that two or three times he had seen a double opening of the duct into the duodenum (Courvoisier, 1890).

² See historical discussion by F. T. Lewis ('12 a) concerning the nature of the hepatic diverticulum.

³ Cadaver no. 6178. White male, age 48. Cause of death, as given in Coroner's report, 'coronary sclerosis.'

for the first time most of the gross anatomical data necessary for adequate interpretation of such a specimen.

As indicated in plate 1, the common hepatic duct splits into two large ducts about 2 cm. above the pars superior of the duodenum. The longer of these, coursing downward obliquely, receives the cystic duct and eventually inserts with the pancreatic duct (*D.panc.*) at the usual site of the ductus choledochus; the shorter duct (*D.hep.ent.*), coursing downward as a direct continuation of the common hepatic duct, inserts on the posterior surface just beneath the summit of the duodenal cap (pars superior duodeni). This I have designated the 'ductus hepato-entericus' on account of its presumptive embryonic origin (p. 84); however, it might be termed an accessory ductus choledochus, for in case of early atresia of the longer duct it would become the only ductus choledochus.

When viewed in situ, the hepato-enteric duct lies immediately anterior to the longer duct, but it has been pictured with the duodenum rotated to the observer's right in order to display its entire course. From its position and its somewhat larger diameter, the anomalous duct would seem to be the chief biliary drainage canal of the liver. But more careful inspection shows that its broad lumen is abruptly occluded at the point where the duct enters the duodenal wall, there remaining only a minute pore through which fluid can be extruded with difficulty (upper of 3 arrows in pl. 1).

Incidentally, it would be of interest to know whether this orifice was closed from the beginning, or whether a stricture developed in post embryonic periods as a response to inflammation or to some idiosyncrasy in the flow of bile. The greater diameter of the hepato-enteric duct would seem to favor the view that it was originally patent. Curiously enough, where there are two gastro-intestinal outlets of the biliary tract, there seems to be a tendency for one of them to become occluded (fig. 6), or to undergo complete atrophy (fig. 1).

In other respects, including the vascular channels at the porta hepatis (*A.hep.*, *V.port.*), the topographical relations of

the biliary tract seem normal. The ampulla of Vater may be identified 8 cm. below the pylorus (lowest arrow, pl. 1); and 2 cm. higher (middle arrow), may be seen the duodenal papilla through which the accessory pancreatic duct emptied.

Lastly, the point of bifurcation of the common hepatic duct in the porta hepatis would seem to indicate a distribution of right and left hepatic ducts comparable to that in the normal liver, i.e., a division of drainage based on a line drawn through the fossa of the gall bladder (McIndoe and Counsellor, '27).

All the available gross data, therefore, coupled with an entire absence of pathology points to a congenital anomaly of the biliary duct system, the peculiar feature being an accessory bile duct connecting the liver with the 'duodenal cap.'

ATYPICAL INSERTION OF BILE DUCTS IN STOMACH

Apparently no specimen quite like the one just described has ever been reported. Yet there are several accounts in the older literature, collected by Paulet (1868) and Courvoisier (1890), which describe bile ducts that empty into the stomach. Thus Paulet writes:

Galen had at first believed, and the Arabs had repeated it after him, that in man one of the biliary ducts ends in the stomach. . . . But this wholly hypothetical assertion of the ancients is found realized sometimes in an abnormal disposition of the termination of the biliary apparatus. Vesale reports that in a sailor the choledochal canal bifurcates and that one of its two branches opens into the stomach. Haller claims that in the cadaver of the Emperor Ferdinand III all the bile was deposited above the pylorus by a single choledochus, but that observation is far from being well established. . . . On the other hand, facts of the same kind reported by Fallope, Vater, Cabrol, Vesling, Bezoldus, and Diemerbroek, present a character certain of authenticity; in all these cases the bile arrived at the pylorus.⁴

⁴ This list should not be accepted without verification, since at least two of the available accounts are merely textbook generalizations. Thus, Vesling writes in 1677: "Now the bile duct (*porus bilarius*) is the passage, a little wider than the cystic duct (*canale vesicae*), which, descending from the liver to the duodenum, distributes the bile to that viscus. . . . For the most part single, sometimes double around the end, rarely it is extended into the very pylorus of the stomach" (Continued on next page.)

Probably the first authentic report in the older literature is that made by Vesalius (1543), incidental to his discussion of the thesis that the usual site of implantation of the bile duct (meatus bilis) "is further removed from the orifice of the stomach than the general run of anatomists supposes." After enumerating the views "discussed from day to day in the schools and in the books of doctors" by those, "however many they are" who "defend themselves only by a mass of authorities, not by inspection of these very things"; and, after affirming that in the whole body, no dissection fell to his lot "more annoying, more difficult than the lower membrane of the omentum and the hollow region of the liver, so much so that any worker, although industrious, easily wanders astray here," he continues:

Then, I noticed that not the smallest portion of the duct of the bile vesicle was extended into the stomach, except in one oarsman of a pontifical trireme. For, as he varied from the make-up of other men in certain organs and especially in the region of the ribs and muscles of the thorax; so, also he possessed a (bile) duct cleft into two branches, of which the smaller or more slender was inserted in the fundus of the stomach (see footnote 4), at the place where the vein, which encircles the right hand portion of that lower place, is first joined to the stomach. This man, indeed, seemed to have been of a hot, dry temperament. However, in order that I might learn whether he had been accustomed to be troubled with vomiting of bile, which Galen declares is frequent in men of this kind, I inquired most diligently from his family whether they had observed in him anything of the sort. And their answer was that they had never seen him vomiting or nauseated even in the biggest storms; indeed as he was very strong, they said he was acclaimed by all because he could blow a trumpet with a very long breath, and because he far surpassed all his comrades with his strong voice and in the numbers he could count in one breath. (From p. 392, book V, chap. VIII, ed. of 1568, Venetiis; copy owned by Prof. C. M. Jackson.)

Similarly, Diemerbroek (1685) concludes his description of a case which emptied into the colon (p. 78) with the following statement: "So also, it has sometimes been observed that a duct is extended from the gall bladder into the pylorus, sometimes into the fundus of the stomach." (A term applied by Vesalius to the lower part of the stomach, which, in the transverse position of the organ, extends well toward the pyloric region, Lewis, '12 b.) "But these are very rare tricks of Nature."

In addition to the case of Vesalius (1543) referred to above, Courvoisier mentions another attributed to *Graseccius* (1605), in which one bile duct passed to the duodenum and the other over the pylorus to the stomach (fig. 9).

In the nineteenth century there are records of two cases in which the only common duct present emptied into the stomach. In view of our knowledge of recent cases, it is quite possible that originally there were two bile ducts in each specimen and that the duodenal member of the pair had atrophied. Thus in *Bleifus'* case (1839) the gall bladder was absent. In this specimen, as described by Budde and by Odermatt, both of whom apparently saw the original account, the exact point where the common duct entered the stomach was not mentioned. Haberland draws a schematic figure and this is copied by Filippini. The patient was a woman 64 years of age who had long suffered from digestive disturbances. In the porta hepatis the common duct was sufficiently dilated to hold a teaspoonful of bile and fine-grained sand. The stomach contained a bile-colored fluid and gall stones the size of peas. Adhesions cemented stomach and duodenum to the liver.

The second of these cases, that of *Laënnec*, is the most extraordinary of all. As reported by Courvoisier and Budde, the common-bile duct inserted into the left side of the stomach just below the cardia. The duct itself was dilated to the thickness of half a thumb and drained the gall bladder and liver. The hepatic ducts were also dilated and both these and the choledochus were filled with round worms (*Ascaris*). Again, Haberland presents a schematic figure.

Fortunately, it is not necessary to depend on these fragmentary older accounts, for recently Filippini ('30) has given us a detailed autopsy report of a modern case (fig. 1), which may be summarized as follows:

Subject, an intensely jaundiced male, 50 years of age. Common-bile duct penetrated pyloric sphincter, just posterior to its summit, and emptied into a depression among superficial undulations of healthy mucosa. Stomach contained bile. Orifice of duct, 1 mm. in

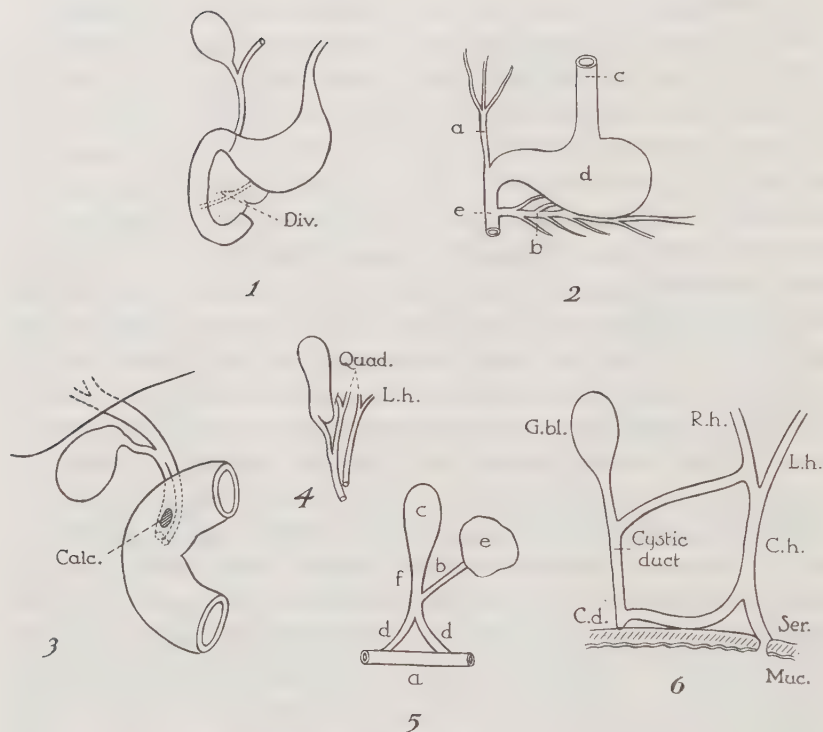


Fig. 1 Filippini's case ('30); jaundiced male, 50 years old; common-bile duct emptying into pylorus (p. 75). Stomach, gall bladder, and bile ducts traced from figure 5 of author. Duodenum and pancreas added schematically from data in author's text. Common-bile duct, 3.5 cm. long, from confluence of cystic and hepatic ducts; orifice of pancreatic duct, 11 cm. below orifice of bile duct. Note diverticulum (*Div.*) on ductus pancreaticus, interpreted by author as remnant of duct of Santorini.

Fig. 2 Blasius' case of 1674 (in woman 40 years of age), illustrating "separate entrance of the pancreatic and bile ducts into the intestine" (p. 78). Traced from figure 1, table 7, of author (ed. 1700). *a*, ductus bilarius; *b*, ductus pancreaticus; *c*, oesophagus; *d*, ventriculus; *e*, intestinum duodenum.

Fig. 3 Kehr's case of 1912 (in woman 36 years of age; p. 80). Traced from figure 63 of author. Ductus choledochus separated into two parallel tubes by septum. Note stone in lateral duct (*Calc.*). Orifices of duct not determined at time of operation.

Fig. 4 Case described by Rodrigues and Adriaão ('31), in 5-month male foetus (p. 80). Drawing reconstructed from gross photograph and description in authors' text. Two ducts separate throughout length. Duodenal orifices of ducts, 1.5 mm. apart; cystic duct, 4 mm. long; common-bile duct, 3 mm. long; hepato-enteric duct, 9 mm. long; quadrate lobe (*Quad.*), supplied by branches from both enteric ducts.

diameter, without trace of valves, but surrounded by ring-like swelling. Common-bile duct, 3.7 cm. long, measured from confluence of cystic and hepatic ducts; dilated to size of little finger. Pressure on gall bladder resulted in egress of yellow bile. Biliary vesicle slightly hydropic; contained 100 cc. yellow bile, but no stones. Liver exhibited suppurative hepatitis with surface nodules and internal abscesses. Dissection of pancreatic duct revealed blind branch (*div.*, fig. 1), vestige of duct of Santorini, in isthmus of gland. Orifice of pancreatic duct in duodenum, 11 cm. below insertion of abnormal choledochus, but not covered with folds that would indicate presence of papilla of Vater. Author presents two alternative explanations: 1) that the primitive hepatic diverticulum detached itself during rotation of duodenum, then later resumed connection with the gastro-intestinal tube in anomalous location (an impossible occurrence, from an embryological standpoint); 2) that it arose by budding from that portion of the intestine that will constitute the future pylorus. In the latter case the author postulates (unnecessarily) that the pancreas must have emptied through its accessory duct.

From this brief survey, it seems quite probable that Filipini's specimen can be interpreted in terms of the writer's case. It is as if the caudal of two common ducts (pl. 1) had atrophied (following failure of its lumen to develop during the solid stage of the biliary tract), thereby leaving the pancreas to drain by itself into the duodenum, and forcing the liver and gall bladder to drain through the more cranially placed duct. Incidentally, development of the pyloric sphincter around the intestinal end of the cranial duct, may have been the principal factor in producing a chronic stasis of the whole biliary tract.

Fig. 5 Blasius' case of '*Porus cholydochus duplex* (1677)'' (p. 79). Traced from figure IV, table VI (ed. of 1700). Common-bile duct, Y-shaped. *a*, intestinali duodeni particula; *b*, ductus hepatici portio; *c*, vesica fellea; *dd*, pori bilarii duplex ramus; *e*, hepatis particula; *f*, ductus cysticus.

Fig. 6 Gentile's case of double bile duct ('31) from jaundiced, 6-week-old female baby (p. 79). Traced from author's figure 1. Gall bladder was of normal size and color, but contained white mucoid material. Pericholangitis and constriction of common bile duct (*C.d.*) at usual site of entrance into duodenum. Ductus hepato-entericus (*C.h.*), patent. Distance between intestinal orifices of two ducts, 3 cm.

ATYPICAL INSERTION OF BILE DUCTS IN DUODENUM

Leaving now, the region of the pylorus, let us examine the cases in the literature which exhibit an abnormally high duodenal origin of the ductus choledochus itself or the presence of bile ducts on the pars descendens of the duodenum. Seven of these cases have been collected from literature preceding the nineteenth century.

Two of them, assigned to *Fallopious* (1606) on the authority of Courvoisier, have already been mentioned.⁵ A third was found by *Diemerbroek* in 1655. In the edition of 1685 he describes this case as follows:

Besides the common duct just mentioned, I demonstrated publicly in the anatomical theatre in the month of April, in the year 1655, a certain other unusual duct, filled with yellow bile and more slender than the ordinary one (which latter, however, was present in the usual manner); and this had nothing in common with the bile duct (porus bilarius) or that which we now call the common duct, but it arose separately, a little above the neck of the gall bladder, in the place where the latter begins to be contracted or narrowed into a neck; and it also was carried separately to the duodenum, into which it was inserted, near the end, at about the width of a finger from the insertion of the other common duct.

In the following year, in another subject, we observed something else that is rare. Next to the usual bile duct (ductus choledochus), we saw, besides, another unusual one extending from the middle of the gall bladder straight into the part of the colon lying next to it.⁶

Following *Diemerbroek*, two cases of abnormal insertion of the common-bile duct were described by *Blasius* (fig. 2).

OBSERVATION IV. Separate Entrance of the Pancreatic and Bile Ducts into the Intestine. On the 26th of February, 1675, I chanced to observe in a newly born foetus that the pancreatic duct had entered the intestine in an entirely distinct place from that which

⁵ See footnote 1.

⁶ Courvoisier cites a similar case by Paw (1656) in which one duct entered the duodenum and the other inserted on the colon; also he mentions the split bile duct described by Dillenius (1715) in which one branch (the obliterated one) went to the duodenum and the other (the patent one) passed into the substance of the pancreas and there broke up into many small branches. The writer concurs with Courvoisier in considering that the first two were probably ulcerative, and not congenital, communications.

belongs to the bile duct, in such a way that the thickness of a finger's breadth might intervene between the place of entrance of the one and that of the other; yet at other times both are accustomed to open by one and the same foramen. In the preceding year a similar example had presented itself in a woman forty years old, where the ducts mentioned had been mutually separated from each other by the distance of a thumb's breadth; this I can demonstrate at my home. See figure I, table VII (compare fig. 2).

From this account it would appear that in the second case the ductus choledochus arose even higher up on the duodenum than the usual position of the accessory pancreatic duct (Santorini), for Blasius describes it as a thumb's breadth (2.5 cm.) above the pancreatic duct (Wirsungii ?). Since the latter usually develops in conjunction with the common-bile duct—and there is no reference to the gall bladder in text or sketch (fig. 2)—one wonders if there were not two common-bile ducts in its earlier development, the lower of which subsequently dropped out. This possibility is suggested by a third case of Blasius portraying a double choledochus in the form of an inverted Y, the fork occurring two fingers' breadth (3.5 cm.) away from the duodenum:

OBSERVATION IX. The Double Choledochal Duct. Just as more than once we found a double pancreatic duct, so also we discovered the bile duct (porus bilarius) or common duct entering the duodenum in a separate place, in such a way that before it entered the intestine, a division occurred at a distance of two fingers. See table VI, fig. IV (compare fig. 5).

The last of the older cases, Vater (1748), as reported by Courvoisier, apparently resembled the one just cited.

Modern cases. In addition to the seven just quoted from the older literature, there are at least seven other instances of double duodenal-bile duct reported in this and the nineteenth century. These will be described briefly in the order of distance of the accessory bile duct from the ampulla of Vater.

Gentile's case ('31), from a 6-week, white female child who died of generalized jaundice, is the most striking (fig. 6). First, the accessory duct inserts as high as 3 cm. above the

true choledochus, the latter being occluded at its usual point of entrance into the intestine. Secondly, the two ducts are connected both proximally and distally, by stout anastomotic channels. Gentile explains this specimen as due to the failure of the liver primordium to elongate normally, so that the proximally undivided stem (the ductus hepato-pancreaticus) was appropriated by the gut, thus effecting two separate entrances of the bile duct into the intestine. But this interpretation does not explain why the orifices of the two ducts are 3 cm. apart (p. 88).

Vetter's case (1803), reported by Courvoisier, is not available for study, but is said by Haberland to resemble Blasius' specimen.⁷

The case of *Rodrigues* and *Adrião* ('31), describing a 5-month male foetus, differs from all others except Diemerbrock's and Amussat's, in that the accessory bile duct is nowhere connected with the choledochus (fig. 4), although the two ducts empty into the duodenum only 1.5 mm. apart.

So also in *Amussat's* case of a 24-year old girl who died of arthritis (as reported by Budde), the two ducts, emptying quite near each other on the duodenum, were entirely separate. But in this specimen the gall bladder was absent.

The next pair of cases, reported by *Kehr* ('13) were observed at operation. Therefore information regarding the termination of the two ducts is uncertain.

In the first patient—a 'Kaufmannsfrau,' 40 years old, operated upon in 1906—the choledochus was described as dividing, immediately under the opening of the cystic duct into two branches. One of these, the size of a little finger, contained a stone as large as a hazelnut, lying just in front of the papilla. The other branch was the size of a goose quill. Both ran parallel and next to one another, and both were thought to empty separately into the duodenum.

In the second patient—a 'Wirtschaftsfräulein,' 36 years old, operated upon in 1912—the choledochus clearly showed a septum and was double. Each part could be probed without

⁷ See page 79.

the two probes touching each other. In the more lateral duct there was a stone (fig. 3). It could not be determined whether each duct opened separately into the duodenum.

The last two cases, *Karpa* ('16) and *Katz* ('30), were both associated with congenital atresia of the duodenum at the

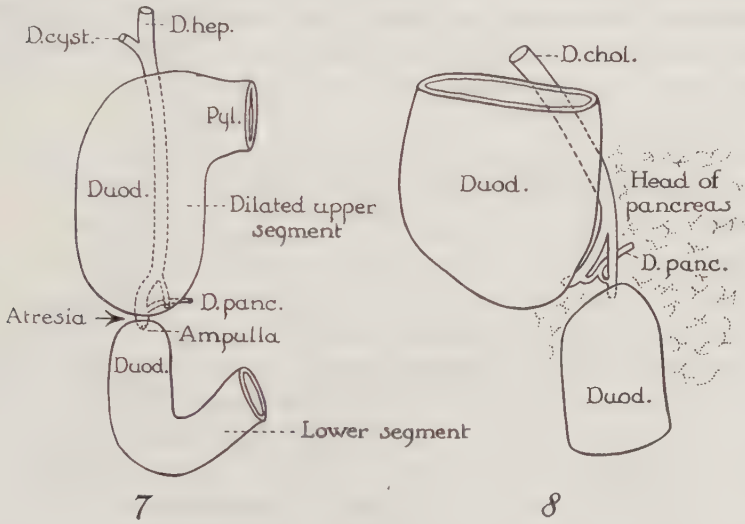


Fig. 7 *Karpa's* case of congenital atresia of duodenum with subdivision of lower end of common-bile duct ('06). (In baby, 4 days old.) Reconstructed schematically from description in authors' text (p. 86). Atresia, 4.5 cm. below pylorus; forking of common-bile duct, 6.5 mm. above orifice of lower duct.

Fig. 8 Case of *Katz* ('30). Congenital atresia of duodenum with subdivision of ductus pancreaticus (*D.pan.*) and lower end of common-bile duct (*D.chol.*). In male baby, 7 days old (p. 86). Reconstructed schematically from gross photograph, photomicrograph, and description in author's text. Death due to inanition and broncho-pneumonia. Gall bladder well filled.

very level of the hepatic diverticulum. In both specimens (new-borns dying a few days after birth) the duodenum was interrupted and the common-bile duct split in two, one-half going with the upper segment of the gut, the other with the lower segment. In the first case (fig. 7) the evidence suggests that the embryonic hepatic diverticulum was divided transversely into upper and lower ducts; in the second case

(fig. 8) a portion of the ventral pancreas was attached to each duct, indicating that the division must have been longitudinal or oblique. At first, Katz suggests that these cases may be explained by double vacuolization of the ductus choledochus, the final separation of the ducts being accomplished by the drawing apart of the two segments of the gut at their point of atresia. But later he raises the question as to whether there were not two primary bile ducts from the very beginning.⁸

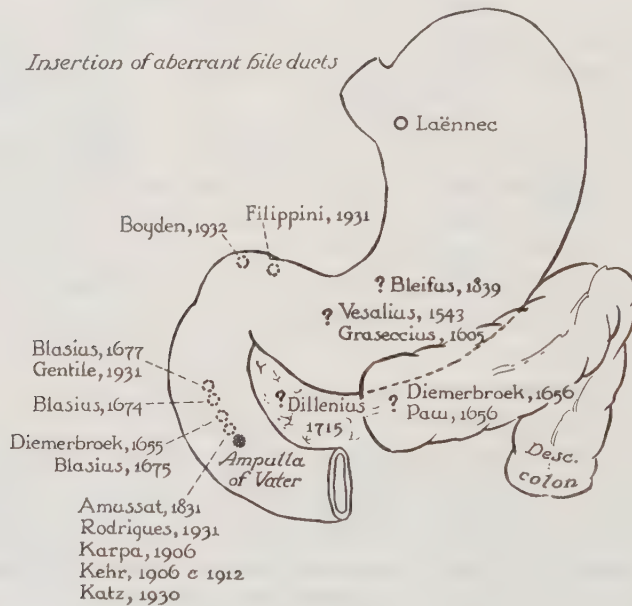


Figure 9

DISCUSSION

To better visualize the distribution of these anomalous structures, the position of each aberrant duct has been plotted upon an outline of the stomach and duodenum (fig. 9). At first glance, this figure recalls the distribution of the accessory pancreas except, perhaps, that aberrant hepatic

⁸ Unfortunately, this quite probable interpretation is based upon the erroneous view that two ducts normally grow out of the duodenum in many mammalian embryos.

ducts are much less numerous, do not occur below the second part of the duodenum, and do not represent small independent masses of glandular tissue. Further inspection, however, reveals the fact that the ducts tend to fall into two general groups—those bordering upon the pylorus or extending further into the stomach (group A) and those (group B) situated on the duodenum within 3 cm. of the usual site of the ampulla of Vater.

Group A. Obviously, those in the first group are the most aberrant types and are therefore the most difficult to explain. At least three possible interpretations may be advanced: first, that the ductus hepato-entericus is an atavistic structure representing a 'throw-back,' as it were, to the double hepatic outgrowths found in reptiles, birds, and some fishes (Mentzer, '29; Katz, '30); secondly, that it is merely a supernumerary outgrowth, analogous to an accessory pancreas; thirdly, that it is due to a chance subdivision of the primary hepatic furrow of the embryo, resulting from some rare disturbance in factors that control the initial phase of development of the biliary duct system.

Opposed to the first interpretation is the dissimilarity of the known cases, hardly any two of them being alike. Furthermore, to duplicate the conditions found in Sauropsida, the two ducts should be much closer together, and not 8 cm. apart, as in the writer's case. Also if this were an atavistic condition, we should expect to find it more commonly in other mammals. But I once had the pleasure of examining 10,000 livers (2500 cats, 2500 calves, 2500 pigs, 2500 lambs) without finding a single case of double choledochus ('26).

A serious objection to the second theory is the fact that no independent masses of hepatic tissue emptying into the gut have ever been reported, so far as I am aware. To be sure, isolated lobes of liver (the so-called *hepar succenturiatum*) have been found on the wall of the gall bladder, ligamentum teres, spleen, and great omentum (Hanser, '30). But with hardly an exception⁹ the position and relations of

⁹Brühl ('26) describes a bean-shaped mass of hepatic tissue in the great omentum near the greater curvature of the stomach and raises the question as to whether the nodule might have been connected with the gastro-intestinal canal.

these lobes identify them as recently detached portions of the liver or, in the case of multiple nodules, as neoplastic formations. Furthermore, the special conditions under which the embryonic liver arises, namely, its intimate relation to vitelline veins, heart, and septum transversum, make such a hypothesis very improbable. To this a priori objection may be added the evidence from experimental embryology which shows that in transplants of the early blastoderms of chick embryos, liver rarely develops in the absence of heart tissue, the two being contiguous when present (Willier and Rawles, '31). Another line of evidence, based upon comparative embryology, is presented by Weissburg ('31), who summarizes the situation as follows:

The liver bay has arisen so early in phylogeny, the hepatogeneous epithelium (arising from it) is so far removed from the intestine proper, that the possibility of the origin of 'accessory livers' within the gut wall itself can no longer be considered . . . the supposition that a double ductus choledochus might have arisen as a double liver anlage, is not confirmed embryologically.

There remains, therefore, the third possibility that the ductus hepato-entericus represents a segregated portion of the primary hepatic furrow, subdivision of the latter having occurred at a time when it was appended to that part of the foregut which was destined to become the stomach.

The setting for such an abnormality exists during the 20- to 24-somite stage of the human embryo. At this period of development the hepatic field lies on the curve of the anterior intestinal portal—a dynamic area between heart, yolk sac, and foregut, itself subject to rapid changes in growth (fig. 11). Here, also, the hepatic furrow is almost surrounded by veins, being flanked on either side by vitelline and umbilical channels and separated from the lung bud above by the sinus venosus. Furthermore, in at least two authentic cases the hepatic furrow is elongated and even subdivided into a pars hepatica and a pars cystica (figs. 10 and 11). In other embryos of this early period, there is no sign of subdivision. In view of this variability of the hepatic

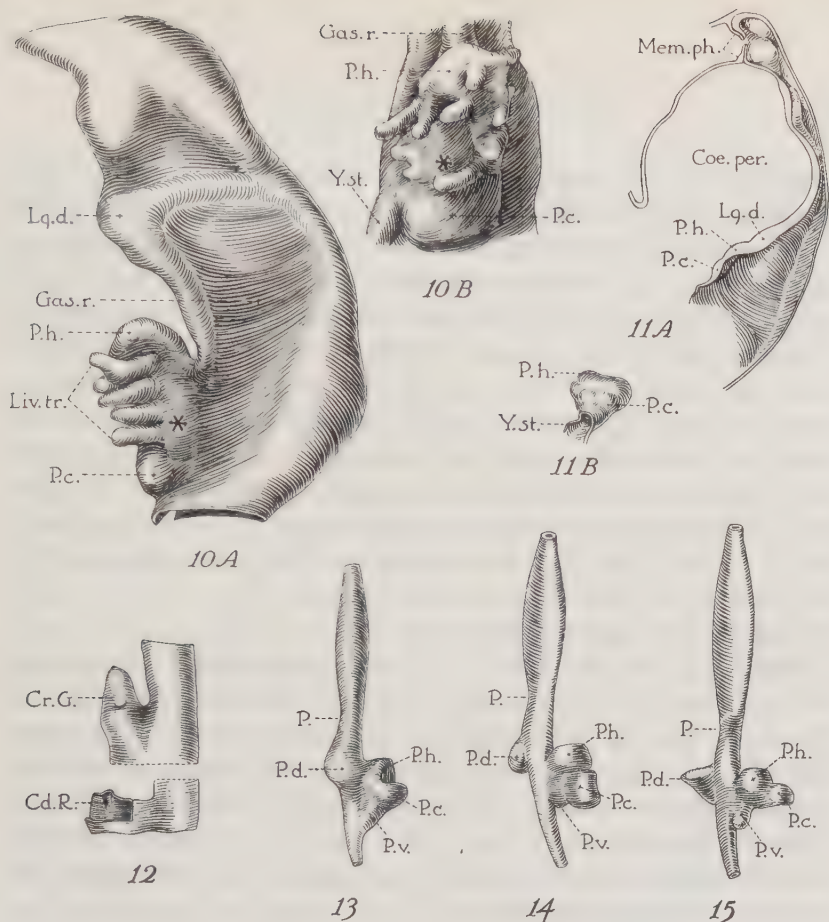


Fig.10 Hepatic diverticulum and foregut in human embryo of 24 somites (Johnson, '17). *A*, view from left side (traced from author's fig. 3, pl. 3); *B*, ventral surface of same (author's fig. 4, pl. 3). *G*, gastric region; *Lg.d.*, lung diverticulum; *Liv.tr.*, liver trabeculae *P.c.*, pars cystica of hepatic diverticulum; *P.h.*, pars hepatica; *Y.st.*, yolk stalk (ant.int. portal). Note precocious differentiation of hepatic diverticulum. Asterisk indicates probable point of division in forming double duodenal ducts. If pars hepatica were a little higher, it might remain attached to the stomach.

Fig.11 Hepatic diverticulum and foregut in human embryo of 20 somites (Davis, '23). *A*, view from left side (traced from author's fig. 23, pl. II); *B*, ventral view of hepatic diverticulum (from author's fig. 24, pl. II). *Coe.per.*, pericardial cavity and heart; *Lg.d.*, primordium of lung; *Mem.ph.*, pharyngeal membrane; *P.c.*, pars cystica of hepatic diverticulum; *P.h.*, pars hepatica.

Fig.12 Detail of foregut of young human embryo, *H.M.*, showing subdivision of hepatic region into cranial duet (*Cr.G.*) and caudal groove (*Cd.R.*). After Felix '22 (fig. 5), viewed from left side. *L*, break in sections.

Fig.13,14,15 Wax models of three stages in development of hepatic diverticulum in human embryos. Viewed from right side (after Pernkopf, '22). Fig. 13, 4.3 mm. embryo; fig. 14, 5.8 mm.; fig. 15, 6.2 mm. *P*, pyloric region; *P.d.*, dorsal pancreas; *P.v.*, ventral pancreas; *P.h.* and *P.c.*, pars hepatica and cystica of liver diverticulum. Figure 14 represents stage at which third type of double duet might be formed.

furrow, it is not unreasonable to infer that once in a few thousand times the pars hepatica should have arisen slightly more cephalad than usual—or have been held there, perhaps, by the aberrant development of anastomoses between right and left vitelline veins; so that when elongation of the gut occurred, most of the pars hepatica would have remained with the gastric portion of the gut while the pars cystica (with adjacent pancreatic and liver fields) was being moved down to its usual site on the duodenum. A fact which seems to favor this hypothesis in the writer's specimen is the presence of a stout distal anastomosis between the two primary ducts (*Anast.*, pl. 1), which suggests that both divisions of the hepatic furrow had grown together distally before the proximal ends had moved apart.

Similarly, the insertion of a single definitive ductus choledochus upon the pylorus or stomach (e.g., cases of Filippini, Amussat, and Laennec) may be explained as due to a somewhat higher origin than usual of the entire hepatic furrow, i.e., just above the zone of growth which separates stomach from duodenum. It is believed, however, that most of these cases represent subdivision of the hepatic diverticulum, the lower duct having atrophied. In this connection it might be pointed out that the normal position of the ampulla of Vater varies greatly in different species of mammals. Thus, in the cat, bile is poured out at the beginning of the duodenum, in man many centimeters below the pylorus.

Group B. Turning, now, to the ducts situated within a few centimeters of the usual site of the ampulla, it is suggested that these may also be explained as due to primary elongation of the hepatic furrow and its precocious separation into pars hepatica and pars cystica (say at asterisk, fig. 10); with the difference that, in this group of cases, both ends of the furrow are located caudad to, the upper one bordering upon, the zone of growth which separates stomach from duodenum (compare also with Felix's subdivided diverticulum, fig. 12). Then, when the duodenum elongates, the two divisions of the furrow would be carried still further

apart, yet would remain on the intestine. Such an origin would best explain the Y-shaped appearance of the two ducts described by Blasius (fig. 5), and the obvious stretching of proximal and distal anastomoses in Gentile's case (fig. 6). Carrying the process a step further, the specimens reported by Rodrigues and Adrião (fig. 4) and by Amussat could be explained as due to separation of the two divisions in a stage comparable to that shown in figure 14.

The last two, however, may fall into the group which I have designated by the name of 'septate ductus choledochus.' This consists of a common-bile duct that is double in part of its course (possibly throughout its course), its two divisions being everywhere parallel and typically adherent (fig. 3; Kehr, '13). Such a type may be explained as formed by double vacuolization during the solid stage in the development of an otherwise normal ductus choledochus. Evidence for this is provided by the existence of at least four embryonic cases ranging from 9 to 22.8 mm. in length, two of which (in 14.5- and 22.8-mm. human embryos) were first reported by F. T. Lewis ('12 a). Each was described as having a single bile duct with a double lumen in the midst of its course, the two cavities being united both proximally and distally. Subsequently, two less striking cases (in 9.0- and 11.0-mm. embryos) were reported by Rietz ('17). With so high an embryonic incidence—2 in 32 embryos (Rietz)—and so low an adult percentage of anomalies, it must be inferred that the septum between the ducts is usually absorbed or is so inconspicuous as to be overlooked. Quite likely, this type is analogous to the bilocular or septate gall bladder occasionally found in man and referable to double vacuolization of the embryonic viscus during the solid stage of its development.¹⁰

¹⁰ This type is not to be confused with double or accessory gall bladders which arise by duplication of the primordium or as accessory outgrowths from hepatic ducts, cyst-hepatic ducts, or hepatic trabeculae bordering the fossa vesicae felleae (Boyden, '26).

At this point it might well be asked why double vacuolization during the solid stage of the choledochus is not sufficient to explain those cases in which two bile ducts enter the duodenum several centimeters apart. The answer is that a lumen first appears in the common duct about the 7.5-mm. stage, at a period when the hepato-pancreatic diverticulum is well down on the duodenum and its area of attachment to the intestine has begun to show relative decrease in diameter; so that ordinarily, if it were cleft, its two halves would not be carried away from one another (see graded series of models by Pernkopf—fig. 13 and older stages). Herein lies the fallacy in Gentile's explanation that "the gut in growing lengthwise appropriated the proximal undivided portions (of the liver primordium), thus effecting two separate entrances of the biliary ducts into the intestine." Surely, if the base of the duct were spreading at such a rate that the pars hepatica were moved 3 cm. up the duodenum, then the ductus pancreaticus (ventral pancreas) should have been moved some distance the other way; yet the entrance of the pancreatic duct into the bowel is described as being normal. To realize how excessive such a translatory growth of the duodenum at the level of the hepatic diverticulum would be, it is only necessary to recall that the maximum width of the intestinal end of the duct in the adult is but a few millimeters.

SUMMARY

Sixteen cases of congenital doubling and five cases of abnormal insertion of the human ductus choleductus are reviewed, in connection with the description of a unique case in which an apparently normal common-bile duct is supplemented by a ductus hepato-entericus that empties into the pars superior of the duodenum.

With the exception of the septate ductus choledochus which is explained as arising by double vacuolization during the solid stage of the embryonic biliary tract, all cases are interpreted on the basis of chance elongation and early subdivision of the primitive hepatic furrow into two divisions corresponding approximately to pars hepatica and pars cystica.

If subdivision occurs very early, in such a way as to leave the pars hepatica above the zone of growth that separates stomach from duodenum, then the pars hepatica will develop into an hepato-enteric duct emptying into the region of the pylorus. Should the division occur below this zone of growth, with the upper diverticulum bordering upon the stomach, then both ducts will empty into the duodenum, but at varying distances from each other. If separation occurs when the furrow is approaching its definitive position, then the two ducts will empty into the duodenum side by side, i.e., one immediately above the other.

Alternative views that represent the double bile duct as an atavistic return to conditions found in Sauropsida, or that attribute it to supernumerary outgrowths analogous to the accessory pancreas, are rejected on grounds of both embryology and comparative anatomy.

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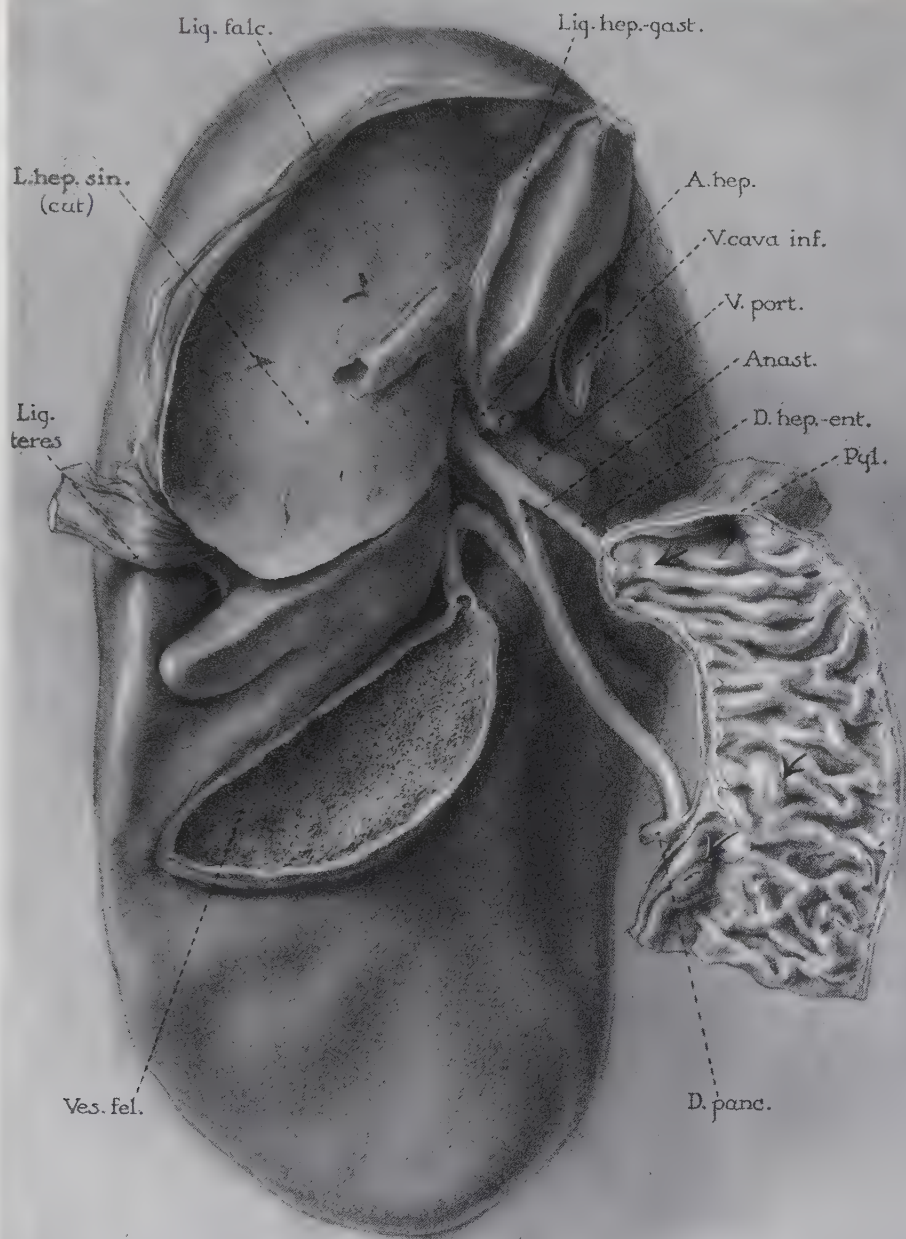
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PLATE 1

EXPLANATION OF FIGURE

16 Liver and duodenum of white male, aged 48, exhibiting duplication of common-bile duct. Left lobe of liver (*L.hep.sin.*) severed and removed at level of falciform ligament (*Lig.falc.*). Same orientation as in figure 140, Cunningham's Manual of Anatomy, vol. 2, 8th ed., '27, except that left wall of duodenum has been removed and the remaining half rotated to observer's right. Common hepatic duct, 4 cm. long; hepato-enteric duct (*D.hep.ent.*), 1.8 cm. long; common-bile duct, 6 cm. long; anastomotic segment (*Anast.*) between common hepatic duct and cystic duct, 1 cm. long; cystic duct, 4 cm. long. Hepato-enteric duct, virtually occluded at point of insertion on pars superior of duodenum; site marked by small pore, 1.5 cm. from pylorus (see uppermost of 3 arrows). Common-bile duct empties low down, on pars descendens, in common with ductus pancreaticus (*D.panc.*), 8 cm. below pylorus (see lowest of 3 arrows). Accessory pancreatic duct opens on duodenal papilla (middle arrow), 2 cm. above ampulla of Vater. Edge of duodenum to observer's right is anterior wall; posterior edge, to which ducts are attached, shows post mortem (?) contracture.



DISTENTION OF THE OVIDUCT IN THE FOWL¹

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TWO PLATES (EIGHT FIGURES)

INTRODUCTION

Ovariectomy in the brown Leghorn brings about striking modifications in primary and secondary sexual characteristics. Among the primary sexual characters profoundly affected by this operation is the oviduct. This organ, which is large, glandular, and convoluted in the laying hen, undergoes a remarkable reduction subsequent to complete bilateral ovariectomy, becoming reduced to a small, straight, flattened tube approximately 2 to 3 mm. in diameter (Domm, '29 a). It also undergoes considerable reduction after sinistral ovariectomy, but was found very variable in such cases, owing to its subsequent activation by a female hormone produced in the hypertrophied right and, when present, also in the regenerated left testis-like gonad (Domm, '27, '29 b, '31). Many of these oviducts have been found to exhibit a rather unusual anomaly, best described as a distention of a part of the oviduct, or frequently the entire oviduct, with a clear watery fluid—a condition which in the mammal is known as hydrosalpinx. This rather unusual phenomenon occurring, with few exceptions, only in the oviducts of ovariectomized fowl has apparently not been described previously, although earlier we called attention to it (Domm, '27, et seq.).

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DESCRIPTION OF CASES

1. Distention of the left oviduct

a. In ovariectomized birds. 1. Cases involving the entire oviduct. Bird no. 727. This bird was sinistrally ovariectomized on August 18, 1925, when 92 days old. Thirteen months later, a laparotomy was performed to determine the completeness of the operation. At this time the anterior part of the oviduct, which is all that could be seen through the lateral incision, was greatly distended with a clear watery fluid. This was drained through a small incision, following which the body cavity was sutured and the bird allowed to recover. A year later (September 29, 1927), it was killed and examined. Post-mortem showed that the oviduct was again enormously distended with a clear watery fluid, measuring 11.4 cm. in length and 6.4 cm. greatest width. It filled the abdominal cavity to such an extent that it is difficult to see how the viscera, which were crowded ventrally forward and to the right, were accommodated at all (fig. 2). The mass was widest posteriorly and gradually tapered anteriorly, as the illustration reveals, and because of its tremendous distention, its walls were very thin and almost transparent. Examination of the anterior end revealed no trace of the infundibulum, this end being completely sealed, rounded, and ending blindly. The cloacal opening was likewise sealed so that no fluid could escape at this point.

Bird no. 988. This bird was sinistrally ovariectomized on May 14, 1927, when 53 days old. It was killed on November 23, 1927. Post-mortem examination revealed a left oviduct greatly distended with a clear watery fluid (fig. 3). It measured 12.2 cm. in length and 3.4 cm. at its widest point. The distention was not as pronounced as that of bird no. 727. However, it will be noticed that the entire oviduct is fairly uniformly distended, with the anterior end bent under somewhat like a hook. The walls of the oviduct are uniformly thin and semi-transparent, with remnants of the severed ventral ligament running diagonally across its surface. No traces of the original infundibular opening are in evidence. This end and the posterior cloacal opening were securely sealed so that no fluid could escape.

Birds nos. 1273, 1275, and 1309. The history of these three cases is very similar and is here introduced primarily because of the early age of ovariectomy and the short period that elapsed in each case between ovariectomy and post-mortem examination.

Birds nos. 1273 and 1275 were sinistrally ovariectomized on July 2, 1928, when 18 days old. Bird no. 1275 was killed on July 23rd, 3 weeks after the operation, while no. 1273 was killed on July 30th, 4 weeks after operation. At autopsy, the entire oviduct in each was distended with fluid. In both, this distention was fairly uniform

throughout, except for a slight constriction anterior to the shell gland region and a slight tapering at the anterior end. Both were securely sealed at either end so that no fluid could escape and neither revealed any trace of the infundibulum. The shape of the distended oviduct of bird no. 1275 was roughly that of an open letter S presumably imposed by ligamentary tension. Its length was 4.8 cm. and its greatest width 0.9 cm. The distended oviduct of bird no. 1273 is shown in figure 3. Its length was 5.2 cm., its greatest width 1.1 cm.

Bird no. 1309 was sinistrally ovariectomized on July 8, 1928, when 10 days old. The bird was killed 5 weeks later. Post-mortem examination revealed an oviduct quite uniformly distended with fluid. In shape it resembled that of bird no. 1273 very closely (fig. 3), although its distention was somewhat less; and both revealed a slight constriction in the region of the shell gland which tended to double the tube on itself. The tube was sealed at either end so that no fluid could escape. It measured 4.3 cm. in length and 0.9 cm. in width.

2. Cases involving only part of the oviduct. Bird no. 1075. This bird was sinistrally ovariectomized on June 2, 1927, when 10 days old. It was found dead on November 21, 1929. Post-mortem examination revealed an enormously distended left oviduct. However, in spite of this tremendous distention, the entire oviduct was not involved. Here approximately the anterior two-thirds was distended to a size about equivalent to the size exhibited by the whole distended oviduct of bird no. 727. The wall of this part was thin and semi-transparent. In addition, the uterine region was distended to approximately the size of a hen's egg with a short, constricted, non-distended region between it and the much larger anterior distended part. Similarly, the posterior end between the distended uterus and its termination at the side of the cloaca was constricted and devoid of fluid. The large distended cephalic part was accidentally punctured, allowing the fluid to escape before it could be photographed. This accident, however, did not allow fluid from the smaller distended uterine region to escape, for even after application of a slight pressure it was retained in this area, showing that the constricted contiguous parts securely sealed the fluid-filled region.

Bird no. 1007. This bird was sinistrally ovariectomized on May 20, 1927, when 11 days old. The bird was killed on December 14, 1928. On post-mortem the anterior third of the oviduct was distended with a clear watery fluid. This distended portion had roughly assumed the size and shape of the testis of a mature cock, measuring 3.6 cm. in length and 1.6 cm. in width. The fluid was securely sealed into this part, for slight pressure failed to dislodge it or force it into the rest of the oviduct posteriorly. Examination revealed no trace of the infundibulum. Caudally, the oviduct was reduced to a small

tube approximately 2 mm. in diameter which could readily be traced to its cloacal termination. The diameter of the shell gland region about 1 cm. in length, was slightly greater, though not distended with fluid.

Bird no. 1257. This bird was sinistrally ovariectomized on June 30, 1928, when 16 days old. When killed, on November 17th, $4\frac{1}{2}$ months after the operation, the anterior two-thirds of the oviduct was filled with fluid, although it was not greatly distended (fig. 5). This part was roughly cigar-shaped, widest in the middle and gradually tapering to a blunt point at both ends. The distended portion was 5.8 cm. long, its greatest width was 1.6 cm. Immediately posterior to this distended region the oviduct was constricted to a narrow tube about 1 cm. in length, following which it revealed another much smaller distended area approximately the size of a kidney bean. The caudal end, approximately 2 cm. in length, was constricted and fluidless.

Bird no. 1310. This bird was sinistrally ovariectomized on July 8, 1928, when 10 days old. It was killed on November 18, $4\frac{1}{3}$ months following the operation. On post-mortem the left oviduct revealed three fluid-filled nodules of nearly equal size separated from one another by short, constricted, fluidless regions (fig. 6). The most posterior fluid-filled nodule was confined to the region of the shell gland and was connected with the cloaca by the short, constricted terminal end of the oviduct. The infundibular end was securely sealed, showing no trace of the infundibulum. The fluid here, as in the above cases, was securely sealed into the regions containing it.

b. In normal birds. Bird no. 970. This bird was hatched on May 21, 1931, and died on March 20, 1932, at the age of 10 months. Inspection of the ovary revealed numerous small follicles, none exceeding a diameter of 4 to 5 mm. The oviduct was uniformly distended with fluid resembling that of bird no. 988 in size and general appearance, although the anterior end was not bent under (fig. 3). This end was smooth and rounded and revealed no trace of the infundibulum. Both ends were securely sealed so that fluid did not escape even under slight pressure.

2. Distention of the rudimentary right oviduct

a. In ovariectomized birds. Bird no. 717. This bird was sinistrally ovariectomized on August 15, 1925, when 89 days old. It was killed on February 27, 1926, 196 days following the operation, at which time the left oviduct had been reduced to a small flattened tube, devoid of fluid-filled areas and having a diameter of 2 to 3 mm. The cephalic end of the rudimentary right oviduct (fig. 7) was greatly distended with a clear watery fluid. This distended portion measured 3.2 cm.

in length and 1.8 cm. in width. It was attached by a narrow, short, constricted portion to a second more caudal part distended with a fluid of the same character, but to a lesser degree, and measuring but 1.0 cm. in length and 0.6 cm. in width. The distended caudal part was attached to the cloaca by the short, constricted, caudal end of the oviduct. No ostial opening could be found. The total length of the oviduct was 5 cm. It was supported throughout its length by a mesentery.

Bird no. 943. This bird was sinistrally ovariectomized on May 3, 1927, when 18 days old. It was killed on April 24, 1928, a few days short of a year subsequent to the operation. At the time of death the left oviduct was large and convoluted, but contained no fluid. The rudimentary right oviduct consisted of a somewhat compressed, marble sized, fluid-filled nodule attached laterally to the cloaca. It revealed no cephalic opening and pressure failed to dislodge the fluid, showing that it was securely sealed.

b. In normal birds. Bird no. 1344. This bird was hatched on August 4, 1929, and killed on February 4, 1930, when 6 months old. The bird at this time was a normal, mature, laying pullet. Post-mortem examination revealed a large ovulating left ovary with follicles of varying sizes. The left oviduct was large, convoluted, and glandular. The right rudimentary oviduct was larger than the average, measuring 4.5 cm. in length. The caudal end was distended with fluid, forming a spherical nodule measuring 1.8 cm. in length by 1.6 cm. in width. The fluid was well sealed into this part, for pressure failed to force it either into the cloaca or into the anterior fluidless part. The oviduct revealed no ostial opening.

Bird no. 1345. This bird was also hatched on August 4, 1929, and was killed on February 26, 1930, when a little less than 7 months old. The bird at this time was a normal mature pullet, revealing a prominent ovary with follicles of varying sizes, the largest approximately the size of a green pea. The left oviduct was large and convoluted, though it had not yet attained the size common for the laying period. The right rudimentary oviduct (fig. 8) was greatly distended with fluid extending from the cloaca forward to the gonad site. Because of its great distention, the viscera were crowded considerably to the left and forward. The total length of the fluid-filled part was 6 cm.; its width, 3 cm. A short constricted caudal end, measuring 1.8 cm. in length, joined it laterally to the cloaca. The fluid could not be dislodged under slight pressure. No traces of an infundibulum were found.

DISCUSSION

The majority of cases exhibiting this phenomenon belong to the second group above described in that only portions of the oviduct were fluid-filled, and of this group approximately twice as many reveal fluid in the anterior or albumen secreting region as reveal it in the posterior shell gland region. The distentions most frequently encountered range in size from that of a small pigeon's egg to that of a large hen's egg. Two of the cases (birds no. 727 and 1075) were exceptional in that the degree of distention was much greater than that usually encountered. Birds carrying such enormously distended oviducts must be tremendously inconvenienced, as there remains little space for the visceral mass, which in such instances is usually crowded laterally and forward. However, bird no. 727 seemed perfectly well when killed and no. 1075 died presumably from an advanced tubercular infection. Cases in which the entire oviduct is distended have probably originated in the albumen secreting region and gradually extended caudally, finally distending the whole tube; or, the albumen secreting region and shell gland may have developed fluid-filled areas simultaneously which, as they increased in size, gradually utilized the intervening tube until they united to form one continuous fluid-filled sac which included the entire oviduct. That this is probable is revealed by cases in which two or more distinct fluid-filled areas occurred which were separated by constricted regions devoid of fluid. Bird no. 1075 described above revealed such a condition. Also the fact that this beaded condition is relatively much more infrequent than cases showing only one fluid-filled area, or nodule, would seem to indicate that they may unite, probably in early stages, forming one continuous fluid-filled area.

The degree of distention revealed does not appear to be determined by the amount of elapsed time since ovariectomy, as cases were found showing small fluid-filled nodules in which the elapsed time since ovariectomy was as great or even greater than that of case no. 727 which showed the

greatest distention we have observed. Bird no. 988 revealed almost the maximum distention observed within 6 months after operation, whereas bird no. 1007 showed but a small fluid-filled nodule 19 months subsequent to the operation.

As is evident from some of the cases described, the juvenile oviduct is as susceptible to the condition as is the more mature oviduct. Bird no. 1275 was ovariectomized when 18 days old and killed 3 weeks after the operation; no. 1273 was the same age as the preceding one when operated, but was killed 4 weeks following the operation, while bird no. 1309 was only 10 days old when ovariectomized and was killed 5 weeks after operation. All three of these cases were ovariectomized very early, yet in spite of this the oviducts in each case revealed pronounced distention with fluid when killed as early as from 3 to 5 weeks subsequent to the operation. When one considers the size of these young individuals, it becomes obvious that the degree of oviduct distention is comparable to that found in the average mature ovariectomized individual.

The frequency with which the condition is encountered is of interest and should be noted. In one experiment involving 180 ovariectomized birds, the condition was found in one form or another in twenty-seven individuals, or 15 per cent of the cases. In another experiment involving a smaller number of birds, the percentage was only a trifle less.

The condition is not entirely confined to the left oviduct of the ovariectomized bird, but may also occur in the rudimentary right oviduct. The cases described are not the only ones in our record in which the rudimentary right oviduct of the ovariectomized fowl revealed this phenomenon. Three other cases (one of which, case no. 717, is here fully described) in which this distention was relatively pronounced were briefly described in a previous publication (Domm, '27, p. 59). While the condition as found in the rudimentary right oviduct differs in no respect from that found in the left, it should, however, be emphasized that its occurrence is relatively much less frequent than in the left oviduct, occurring in less than 1 per cent of our cases.

We have thus far encountered only two cases in which the left oviduct of the normal bird revealed a comparable condition. In both instances the birds were 10 months old at the time of death, both revealed about the same degree of distention, and in each the entire oviduct was involved.

In the rudimentary right oviduct of the normal bird only three cases have so far come to our attention. In two of these (nos. 1344 and 1345) above described the birds were normal adult pullets, which had never been used for experimental purposes, while the third case was that of a bird in which the left oviduct had been removed. In this instance the cephalic end was distended to approximately the size of a hen's egg and was attached to the side of the cloaca by the short, constricted, caudal end.

The question quite naturally arises as to the condition responsible for this unusual anomaly. So far as we know, no one has ever described or referred to the condition in ovariectomized fowl and we know of no reference to its occurrence in the normal. In casting about for probable causes, we naturally come to the conclusion that it is in some manner associated with the involution of the oviduct due to ovarian inactivity or removal. It is altogether conceivable that the fluid found in the oviduct in these cases is the product of glands within its walls. It is probable that such a fluid is continually forming, particularly during the active cycle, but that under ordinary conditions it passes to the exterior or into the body cavity. Why, then, should it not have been eliminated in the same manner in the cases described instead of becoming imprisoned? The reason for this, we believe, may be partly found in our method of operation, in preparation for which all membranes are carefully removed to expose the ovary. In doing so the ostium must invariably be destroyed, and as a result this end later is frequently sealed. The narrow cloacal opening is probably invariably sealed due to the constriction and shrinkage of the degenerating oviduct. Having both ends of the oviduct sealed, secreted fluids could quite naturally be retained. If we assume absence or imper-

fect development of cephalic and caudal openings, the same explanation will apply to cases found in the normal hen.

The condition as revealed by the rudimentary right oviduct quite readily lends itself to the same explanation. While the right oviduct very seldom is normal in size and structure, it by no means follows that it is always an insignificant, non-glandular, cloacal stub. On the contrary, it not infrequently attains a length of from 4 to 6 cm. and may reveal glandular development showing response to female hormone. Ostial and cloacal openings are usually difficult to demonstrate. Thus, if absence of opening leads to fluid distention during periods of oviducal involution, then it is only logical to suppose that the rudimentary right oviduct when glandular and devoid of openings may be subject to the same condition.

Another anomaly of the oviduct which bears some resemblance to the condition just described is one in which the oviduct is found distended with a hard coagulated substance (fig. 1). This condition occurs in but a small percentage of our ovariectomized fowl and was also previously mentioned (Domm, '27). We have never observed it in other than ovariectomized birds, and here only in the left oviduct. Such oviducts more rarely reveal the pronounced distention shown by some of the fluid-filled cases, the case illustrated being exceptionally large. These cases also are frequently less uniform in outline than are the fluid-filled ones. Some of them bear a striking similarity to the egg-bound oviduct of the laying hen, but obviously their formation cannot be attributed to this cause, since all traces of ovary were removed usually long before the first laying period. In our experience the condition has most frequently involved but a part of the oviduct, the lumen of which was distended with a firm, homogeneous, non-granular substance. It was early felt that this peculiarity was a direct out-growth of the fluid-filled condition above described; that by the ultimate coagulation and hardening of the fluid contained within the oviduct such a condition might be produced. The condition in some cases, however, may be a pathological one.

SUMMARY

1. This article records observations on cases in which the oviduct of the brown Leghorn fowl became distended to varying degrees with a clear watery fluid.

2. Representative cases are described in which the whole, or a portion, of the left oviduct of the ovariectomized individual became distended.

3. Two cases were found, one of which is described, in which the left oviduct of the normal hen revealed the condition.

4. Cases are also described in which the same condition was revealed by the cloacal remnant of the rudimentary right oviduct in the ovariectomized as well as the normal individual.

5. The condition was encountered in the left oviduct 3 weeks to several years following ovariectomy regardless of the age at the time of operation.

6. The anomaly was found in approximately 15 per cent of the cases in the left oviduct and in less than 1 per cent of the cases in the rudimentary right oviduct of ovariectomized individuals.

7. The condition is apparently much less frequent in normal individuals. We found only two cases in which the left oviduct, and three in which the rudimentary right oviduct, revealed the condition.

8. Individuals showing the condition were typical in other respects. All ovariectomized birds were found to have had satisfactory operations and had developed as typical poulards.

9. It is believed that the anomaly is associated with degeneration or involution of the oviduct and that the imprisoned fluid is a product of its glandular walls.

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PLATE 1

EXPLANATION OF FIGURES

1 Distention of the entire left oviduct. This case differs from all the others in that the oviduct is distended with a firm homogeneous substance and not with fluid. It is illustrated because of its striking similarity to the fluid-filled condition described in this paper. The bird was hatched on June 23, 1926, and sinistrally ovariectomized on August 13, 1926, when 51 days old. It was killed on December 9, 1930, 4 years and approximately 4 months following the operation. The bird had been sickly for some months prior to the time it was killed; necropsy revealed the tremendously distended oviduct illustrated. Other organs were normal and revealed no evidence of disease. The visceral mass was crowded forward ventrally and to the right. The body wall was ruptured ventrally on the right side between the third and fourth ribs, allowing a loop of the intestine to come through and form a prominent nodule under the skin. *C.*, caecum; *Cl.op.*, cloacal opening; *Ov'd.*, left oviduct.

2 Distention of the entire left oviduct. This bird was hatched on May 18, 1925, and sinistrally ovariectomized on August 18, 1925, when 92 days old. The bird was killed on September 29, 1927, 2 years and 42 days following the operation. Labels as in legend for figure 1.

3 Distention of the entire left oviduct. This bird was hatched on March 22, 1927, and sinistrally ovariectomized on May 14, 1927, when 53 days old. The bird was killed on November 23, 1927, 193 days following the operation. *Cl.op.*, cloacal opening; *Ov'd.*, left oviduct; *R.*, rectum.

4 Distention of the entire left oviduct. This bird was hatched on June 14, 1928, and sinistrally ovariectomized on July 2, 1928, when 18 days old. The bird was killed on July 30th, 28 days after the operation. *Cl.*, cloaca; *Ov'd.*, left oviduct; *Ur.*, ureter. Testis-like right gonad removed for study.

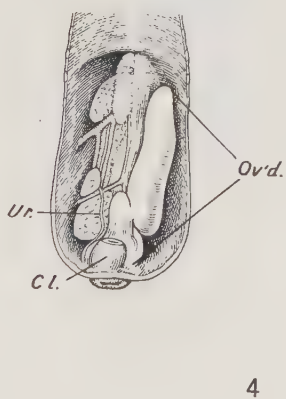
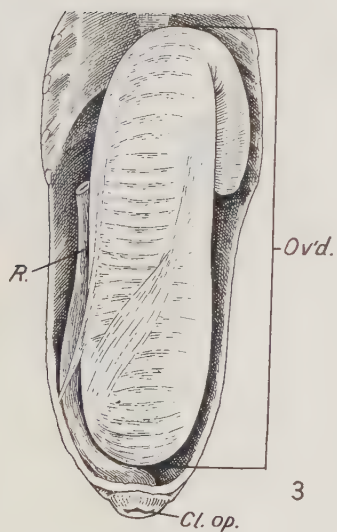
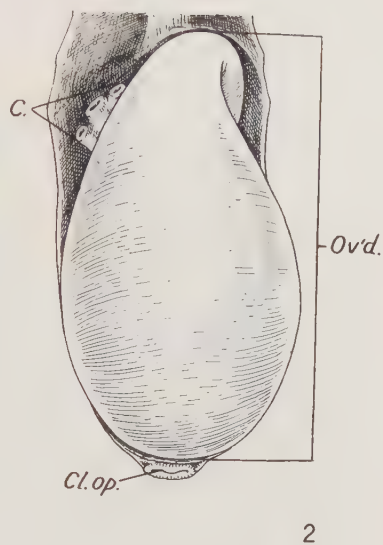
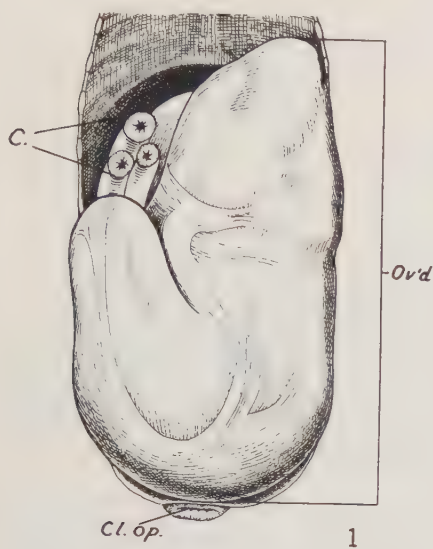


PLATE 2

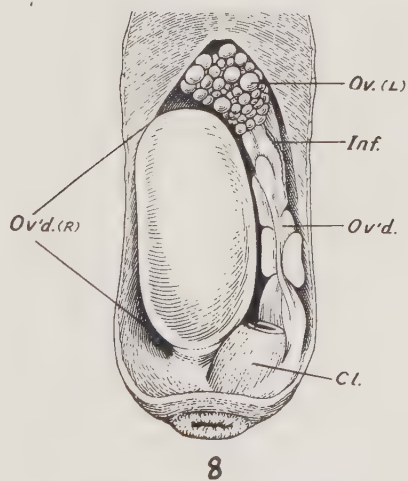
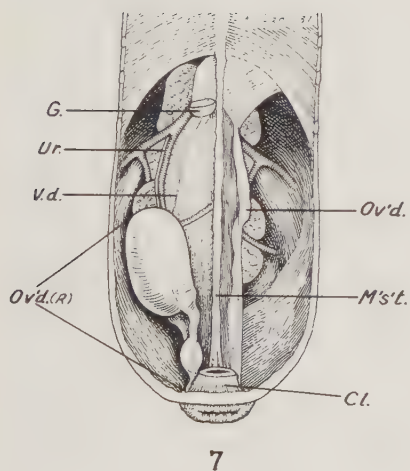
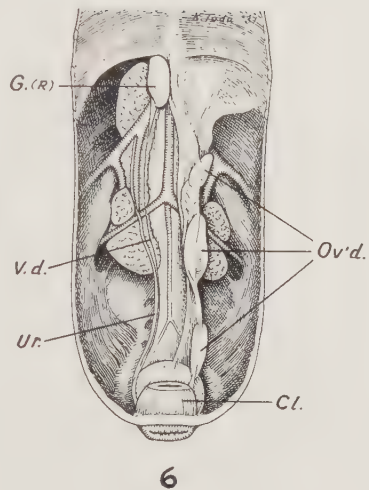
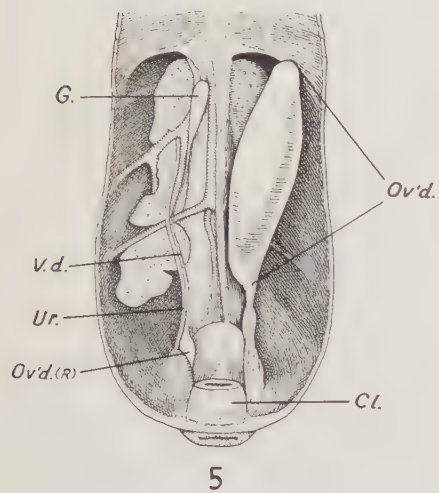
EXPLANATION OF FIGURES

5 Distention of a portion of the left oviduct. This bird was hatched on June 14, 1928, and sinistrally ovariectomized on June 30, 1928, when 16 days old. The bird was killed on November 17, 1928, 140 days following the operation. *Cl.*, cloaca; *G.*, right testis-like gonad; *Ov'd.*, anterior fluid-filled portion of left oviduct; *Ov'd (R)*, remnant of right rudimentary gonad; *Ur.*, ureter; *V.d.*, ductus deferens.

6 Distention of a portion of the left oviduct. Case revealing multiple distentions. This bird was hatched on June 28, 1928, and sinistrally ovariectomized on July 8, 1928, when 10 days old. The bird was killed on November 18, 1928, 133 days following the operation. *Ov'd.*, fluid-filled parts of left oviduct. Other labels as in legend for figure 5.

7 Distention of the right rudimentary oviduct in an ovariectomized fowl. This bird was hatched on May 18, 1925, and sinistrally ovariectomized on August 15, 1925, when 89 days old. The bird was killed on February 27, 1926, 196 days following the operation. *Cl.*, cloaca; *G.*, posterior end of right testis-like gonad (rest removed for section); *M's't.*, median dorsal mesentery; *Ov'd.*, left oviduct; *Ov'd. (R)*, fluid-filled right rudimentary oviduct; *Ur.*, ureter; *V.d.*, ductus deferens.

8 Distention of the right rudimentary oviduct in a normal fowl. This bird was hatched on August 4, 1929, and was killed on February 26, 1930, when 206 days old. *Inf.*, infundibulum; *Ov. (L)*, left ovary. Other labels as in legend for figure 7.



ON THE UNMYELINATED FIBERS IN THE SPINAL NERVES

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ONE PLATE (FOUR FIGURES)

The existence of non-myelinated fibers in the peripheral nerves was pointed out by Sherrington (1894), and in 1911 Ranson demonstrated their presence in the spinal nerves, using the pyridine-silver method of staining. He showed ('15) that they were chiefly confined to the cutaneous nerves, but some were also to be found in the motor divisions. The contribution of unmyelinated fibers through the ventral spinal root is small (Ranson, '12). In a recent publication in collaboration with Miss Davenport ('31), Ranson has shown that these non-myelinated fibers in the spinal nerves are not all sympathetic in nature. In six cats they removed the right abdominal sympathetic chain from the diaphragm to the pelvis. These animals were allowed to live for periods ranging from 39 to 84 days to allow for complete degeneration of all of the sympathetic fibers entering the spinal nerves through the gray rami. The saphenous nerve was particularly selected as being purely cutaneous, and adjacent portions of the nerve were stained by osmic acid and the pyridine silver technique, respectively. The sections on the right side, from which all sympathetic fibers had been removed, were seen to contain a very large number of unmyelinated fibers, stained darkly by the pyridine-silver technique, but not showing in the corresponding adjacent section stained by osmic acid.

Ranson and Davenport ('31) conclude that these fibers arise from small unipolar cells in the spinal ganglia, which Ranson

had previously ('12) shown to give rise to unmyelinated axons which divide into central and peripheral branches. Langley ('22) considered that very few, if any, non-myelinated fibers enter the spinal cord in the posterior roots, but he did not adopt any specific stain for nerve axons.

Ranson ('15) also considers that these unmyelinated nerves serve for the conduction of impulses giving rise to protopathic sensation. He bases this on the following grounds:

1. A certain correlation between the sharpness of epicritic sensation and the proportion of myelinated fibers in the nerve distributed to that particular area.

2. Rate of regeneration. Following division and primary suture of a nerve, the innervation returns to the sweat glands and blood vessels (post-ganglionic non-myelinated fibers) about the same time as protopathic sensation is restored.

3. Head has contended that the viscera (e.g., stomach) possess protopathic, but not epicritic sensibility, and the main sensory innervation of the stomach is with unmyelinated fibers.

4. The unmyelinated fibers of the dorsal root enter the spinal cord, according to Ranson ('13) and turn into Lissauer's tract, which consists chiefly of unmyelinated axons, scattered among which are a few myelinated fibers. The individual fibers run only for a very short distance and then end around cells in the substantia gelatinosa. This seems to be in accordance with the intraspinal pathway for impulses of protopathic nature, particularly painful afferent impulses.

The evidence appears suggestive, but is circumstantial rather than conclusive. Head's hypothesis of the protopathic sensibility of the viscera is not universally accepted: it has now been shown that the viscera do not respond to any of the ordinary means of mechanical stimulation, but are sensitive to the adequate stimulus, which appears to be muscular contraction of the visceral wall producing tension. However, as Ranson points out, the view of the unmyelinated nerve fibers subserving protopathic sensibility is put forward as a working hypothesis.

Through the kindness of Professor Ranson in sending some of his original sections to Professor Stopford, I have had the opportunity of examining these personally, and the present paper is simply in confirmation of the findings of Ranson and Davenport. Both abdominal sympathetic chains were, however, extirpated in these experiments to exclude any possibility of fibers crossing from the opposite side. The two lumbar sympathetic cords in the cat lie in very close apposition. Four cats were used in these experiments, and by an abdominal incision through the linea alba, a bilateral lumbar sympathectomy was performed, the ganglionated chain being in each case removed in one long stretch. In all the completeness of the extirpation was confirmed by postmortem examination. The animals were allowed to live for 6 to 7 weeks to allow for complete degeneration of all the sympathetic fibers to the lower limbs. The long saphenous nerve was selected, as recommended by Ranson and Davenport, and the technique of fixing and staining as given by them (pp. 332 to 334) was carried out in every detail.

The results are seen in the accompanying microphotographs. Small darkly staining nerve fibers can be seen in the sections stained by the pyridine silver method which do not show on the osmic acid preparations. Counts of the transversely cut sections of the nerve fibers were made in the corresponding osmic and pyridine silver preparations. These showed almost three times as many fibers in the silver as in the osmic stained sections.

	OSMIC ACID	PYRIDINE SILVER		
	Total number of fibers	Total	Myelinated	Unmyelinated
Cat 2. Left, saphenous nerve (one fascicle)	402	1615	426	1189
Cat 3. Left saphenous nerve (one fascicle)	382	1400	372	1028

DISCUSSION

These findings are in entire agreement with those of Ranson and Davenport. The appearance of the unmyelinated fibers, both in the longitudinal and transverse sections, is convincing. There can be no doubt as to their existence, and from the degeneration experiments the sympathetic origin of such fibers is excluded. Kiss ('32) has expressed the opinion that they are axons of sympathetic multipolar cells situated in the posterior-root ganglia, but gives no evidence for such a statement. It seems difficult to see why the multipolar cells that Kiss recognizes in the spinal ganglia should be designated as part of the sympathetic nervous system.

But the evidence recently brought forward by Kuré and his collaborators ('27-'31) on what they term the 'spinal para-sympathetic system' seems to have a possible bearing on the significance of the unmyelinated nerve fibers in the spinal nerves. They have shown by a series of degeneration experiments that there exists in the posterior roots many efferent finely medullated nerve fibers which end around cells in the spinal ganglion. From these cells the post-ganglionic fibers, which apparently may be finely myelinated or unmyelinated, pass peripherally into the mixed spinal nerve and, according to Kuré, serve for trophic, secretory (sweat), and vasomotor effects. Though some of their conclusions are still unacceptable, the evidence is strongly in favor of the existence of such pathways. Is it not possible that some of the unmyelinated nerve fibers in the spinal nerves are part of this 'spinal para-sympathetic system'?

SUMMARY

1. The evidence for the presence and probable functions of the non-medullated fibers in the spinal nerves has been briefly reviewed.
2. The non-sympathetic origin of many of these fibers has been confirmed.
3. An explanation of a possible relationship between these non-myelinated fibers and the spinal parasympathetic system is put forward.

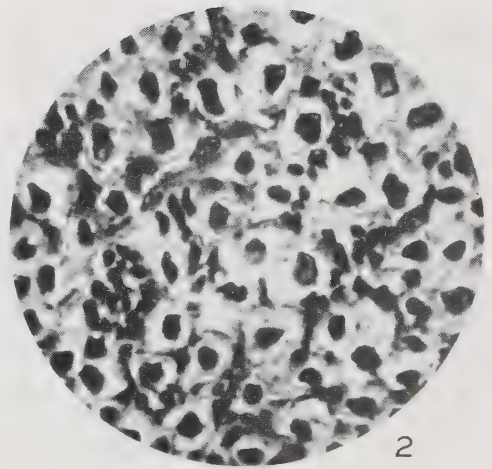
My grateful thanks are due to Prof. J. S. B. Stopford, who has been ever ready with his help and advice and who has kindly allowed me to carry out this work in his department.

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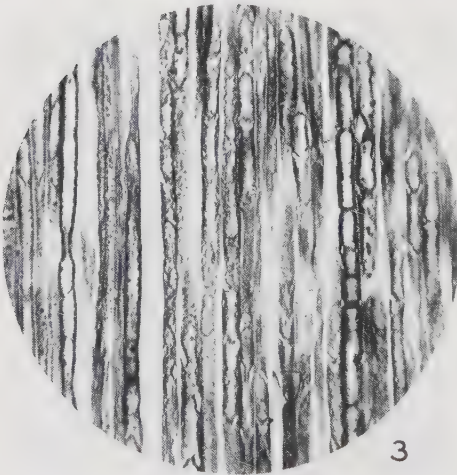
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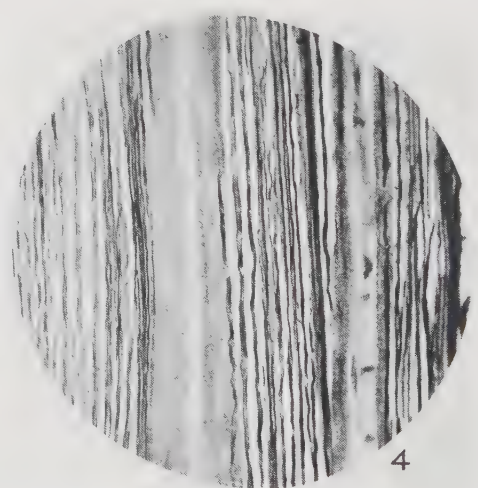
1



2



3



4

1 and 2 Transverse sections of long saphenous nerve of cat following bilateral lumbar sympathectomy. Stained osmic acid and pyridine silver, respectively. $\times 1200$.

3 and 4 Longitudinal sections of same. Stained osmic acid and pyridine silver, respectively. $\times 250$.

AN EXPERIMENTAL STUDY OF THE SENSORY NEURONES IN THE VENTRAL SPINAL NERVE ROOTS OF THE CAT¹

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INTRODUCTION

Although the ventral spinal nerve roots are usually considered to be composed entirely of efferent neurones whose cell bodies are contained within the central gray matter, a number of observations indicating that there are exceptions to the rule have been recorded. The physiological experiments of Claude Bernard (1858) determined that, in dogs, pain responses can be elicited by stimulating the ventral roots when the dorsal ones are intact. Correlatively, not all myelinated nerve fibers degenerate in the frog, cat, monkey, and dog after sectioning the ventral roots near the spinal cord (Dunn, '14; Sherrington, 1894; Windle, '31). Nerve cells of the sensory type have been observed in ventral roots of many different species of animals, including man (Freud, 1878; Schäfer, 1881; Onodi, 1885; Hoche, 1892; Tanzi, 1893; Kölliker, 1894; Zacharriades, 1896; Piolti, '30 a and b; Windle, '31), but most of the data are little known, because they were recorded in obscure places. To our knowledge, the course taken by the processes of ventral root cells is entirely unknown.

Recently, clinical and physiological observations (summarized by Wartenberg, '28) have led various investigators to postulate the presence of an accessory pathway in the ventral roots for conduction of painful impulses after dorsal root section in man (Knapp, '08; Lehman, '24; Foerster,

¹ Contribution no. 178.

Altenburger, and Kroll, '29). Some of this evidence is counterbalanced by other clinical studies that show complete relief from pain or complete loss of all sensations following similar operations (reviewed by Davis and Pollock, '30). The physiological studies of Shaw ('24) are sometimes cited in support of such a hypothetical pathway; but this investigator's percentage of operative failures and the uncertain criteria used to determine sensory responses in his animals throw considerable doubt upon his results. The experiments of Claude Bernard (1858) do not indicate that pain may be conducted into the spinal cord over the ventral nerve root. It is important to determine whether or not the sensory neurones contained in the ventral roots—and ignored by nearly everyone who has suggested a theoretical centripetal ventral root conduction route—send processes into the spinal nerves. The effects produced in these sensory elements by sectioning the nerves to one hind leg in the cat are herewith reported.

MATERIAL AND METHODS

Seven adult cats were operated aseptically under 'Nembutal' anesthesia. The abdomen was opened in the median line, in each case, the genitofemoral, femoral, obturator, and sciatic nerves were sectioned unilaterally as close as possible to the places where they are formed. Frequently, the roots of the plexus which contribute to these nerves, rather than the chief trunks, were cut; other branches of the plexus were rendered functionless in these specimens.

Table 1 summarizes the material obtained from these operated animals. Ventral roots on the normal as well as the operated side were removed by cutting them at their junction with the dorsal roots and stripping them away from the spinal cord. During the procedure the tissues were kept moistened with physiological saline solution. Each root, straightened out carefully on a small piece of glass, was immersed in a separate vial of van Gehuchten's fixing fluid where it remained for 15 to 24 hours. All were stained lightly

with eosin, to render them visible in the paraffin block, and sectioned longitudinally. A 1 per cent aqueous solution of toluidin blue was used to color the chromatic substance.

TABLE 1
Summary of material used for the present study

CAT NO.	DAYS INTERVAL BETWEEN OPERATION AND AUTOPSY	VENTRAL ROOTS REMOVED AT AUTOPSY								
		Lumbar					Sacral			
		3	4	5	6	7	1	2	3	
1	14	*	*	*	*	*	*	*		
2	11		*	*	*	*	*	*	*	
3	11		*	*	*	*	*	*	*	
4	13		*	*	*	*	*	*	*	
5	13		*	*	*	*	*	*	*	
6	14		*	*	*	*	*	*	*	
7	12		*	*	*	*	*	*	*	

RESULTS

Three thousand and ninety-nine sensory type nerve cells were counted in the ventral roots of both sides. An examination of table 2 shows that their distribution varies greatly in different roots as well as in different cats. In animal no. 5, only 71 cells were found; but 1247 cells were encountered on the two sides of no. 3. Although many roots contained no cells at all, more than 100 were observed in several and 275 were enumerated in one of them. They occur singly, in rows, or in little ganglia and are found in all parts of the ventral roots. They are especially numerous near the junctions with the sensory ganglia. Their shape depends upon whether the cells are placed in clusters or singly among the nerve fibers of the ventral roots. In the latter position they tend to be elongate ovoids.

All sizes and types of cells found in the dorsal root ganglia occur in the ventral roots, but small ones are very numerous and large cells are relatively few. When one classifies them according to three main types (Sheinin, '30), the majority fall into the 'C' group, which consists of small and medium sized cells containing coarse Nissl granules. The granules are not evenly scattered throughout the cytoplasm, but

TABLE 2

Summary of results: Number and percentage of normal, doubtful, and chromatolyzed cells in the ventral roots of each cat¹

CAT NUMBER		1			2			3			4			5			6			7		
		N.	D.	C.	N.	D.	C.	N.	D.	C.	N.	D.	C.	N.	D.	C.	N.	D.	C.	N.	D.	C.
a. Unoperated control sides																						
Root L 4		0	0	0	39	68	2	68	15	1	32	10	1	15	0	0	72	10	0	13	1	0
Root L 5		1	0	0	8	2	0	95	32	3	22	1	0	18	0	0	0	0	0	101	12	2
Root L 6		0	0	0	1	0	0	49	9	0	7	5	0	3	0	1	1	0	0	13	5	0
Root L 7		1	0	0	0	0	0	2	0	0	0	0	0	2	2	0	0	0	0	14	0	0
Root S 1		7	9	2	0	0	0	3	0	0	4	5	0	0	0	0	0	0	0	0	0	0
Root S 2		79	74	6	3	0	0	95	31	4	7	11	0	0	3	0	98	42	10	91	41	8
Root S 3		x	x	x	6	1	0	205	64	6	93	3	0	13	0.	0	0	0	0	0	0	0
Totals		88	83	8	57	71	2	517	151	14	165	35	1	51	5	1	171	52	10	232	59	10
Percentage of N.D.C. in each cat		49.2-46.4-4.5-			43.8+54.6+1.5+			75.8+22.1+2.1-			82.1-17.4+0.5-			89.5-8.8-1.8-			73.4-22.3+4.2-			77.1-19.6+3.3+		
b. Operated sides																						
Root L 4		0	0	0	5	0	0	142	17	5	0	0	0	0	0	0	0	0	0	0	0	0
Root L 5		0	0	0	3	2	0	93	26	0	25	5	1	0	0	0	0	0	0	76	5	1
Root L 6		0	0	0	0	0	0	23	17	1	12	2	1	0	0	0	0	0	0	27	3	2
Root L 7		0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Root S 1		0	0	0	0	0	0	3	2	0	46	28	1	1	0	0	56	50	32	0	0	0
Root S 2		0	0	0	17	0	0	68	41	4	62	44	9	6	4	0	61	32	7	41	35	20
Root S 3		x	x	x	6	3	0	82	35	4	3	2	0	2	1	0	6	6	0	0	0	0
Totals		0	0	0	31	5	0	412	139	14	148	81	12	9	5	0	123	88	39	144	43	23
Percentage of N.D.C. in each cat					86.1+13.9-			72.9+24.6+2.5-			61.4+33.6+5.0-			64.3-35.7+0			49.235.215.6			69.3+20.010.7-		

¹ The letters N., D., and C. at the head of the column of figures indicate normal, doubtful, and chromatolyzed cells. The letter x means: absence of observations.

normally accumulate at the periphery or around the nucleus. The nucleus is not infrequently eccentric. Many medium sized type 'A' cells are encountered. The Nissl substance in these is relatively finely divided and scattered evenly throughout the cytoplasm. The nucleus is usually placed near the center of the cell. Type 'D' cells, which are very small ones with scanty dark or cloudy cytoplasm and practically no Nissl granules, occur occasionally.

Many cells are located close to the periphery of the root and often present abnormal appearances like those at the periphery of spinal ganglion preparations. Exposure to air in the interval between opening the vertebral canal and removing the roots or compression of the nerves against the glass slides before fixation may have produced some damage to these cells. In general, abnormal forms occur with greater frequency in ventral roots than in dorsal root ganglia.

The criteria upon which judgment of normal and chromatolyzed cells was based were formulated as follows: *Normal cells* are all those in which the nucleus occupies a fairly central position and presents an even membrane, in which chromatic substance is in the form of distinct granules which are not fragmented and dispersed, and in which no marked shrinkage or swelling can be observed. A cell is considered to be *chromatolyzed* when the nucleus is eccentric and shows a darkly stained thickening of its membrane, when swelling is evident, and when fragmentation and dispersion of the cytoplasmic chromatic substance and clouding of the, otherwise clear, background is distinct.

Many cells could not be considered normal, nor did they fulfill the requirements of chromatolysis. Some type 'A,' as well as 'C,' cells lacked true dissolution of Nissl substance, but were vacuolated; others exhibited marked nuclear displacement and slight swelling; still others, in which injury in handling the tissues or some other fault in the technique produced severe artifacts of the cytoplasm or nucleus, could not be classified as normal nor could they be said to show the axone reaction. Therefore, a third group—designated *doubt-*

ful—was formed. Practically all type 'D' and many type 'C' cells, in which Nissl granule characteristics are normally often similar to those that follow axone injury, were included in this group.

We have striven to be conservative in judgment, but have favored placing every cell, in which three of the principal characteristics of chromatolysis—even in small degree—are present, in the altered group. When the material was prepared, the slides were numbered in such a way that, at the time of examination, we did not know whether we were dealing with operated or control specimens. This remained unknown until all counts were completed. Consequently, factors of personal bias have been eliminated to a great extent.

The results of classification of ventral root neurones are summarized in tables 2 and 3. A greater average number of all cells occurred on the unoperated than on the operated side. The maximum amount of chromatolysis observed on the normal side of any one cat was 4.5 per cent. That on the operated side was 15.6 per cent and 10.7 per cent in two cases; but other cats contained no chromatolyzed nerve cells. These figures would not be altered appreciably if we disregard the fourth lumbar and third sacral roots which do not contribute as heavily to the nerve supply of the leg as others. A comparison of table 3 a and b shows a mean decrease of 5.9 per cent in normal cells and an increase of 4.1 per cent in chromatolyzed ones on the operated sides.

DISCUSSION

What is the evidence in favor of an accessory pain conduction pathway by way of the ventral spinal nerve roots? If the results of the present experiments are viewed collectively (table 3), very little evidence can be found. A difference of 4.1 per cent chromatolyzed cells in favor of the operated side is not very convincing. However, when the results obtained on individual animals are examined (table 2), differences of 11.4 and 8.2 per cent are found. When 15.6 per cent of the corresponding ventral root afferent neurones show the axone

reaction after sectioning the nerves to one leg (cat 6), one dare not deny the possibility that a significant number of cells whose peripheral processes course in the spinal nerves may occasionally exist in some of the ventral roots! Of course, their central processes may not enter the spinal cord over the ventral roots, but may course back to the junction of the two roots and proceed over the dorsal ones.

TABLE 3
a. Summary of the unoperated sides

CAT NO.	TOTAL	NORMAL	DOUBTFUL	CHROMATOLYZED
1	179	88	83	8
2	130	57	71	2
3	682	517	151	14
4	201	165	35	1
5	57	51	5	1
6	233	171	52	10
7	301	232	59	10
Totals	1783	1281	456	46
Per cent	100	71.8+	25.6-	2.6-

b. Summary of the operated side

1	0	0	0	0
2	36	31	5	0
3	565	412	139	14
4	241	148	81	12
5	14	9	5	0
6	250	123	88	39
7	210	144	43	23
Totals	1316	867	360	88
Per cent	100	65.9+	27.4-	6.7-

The number of chromatolyzed cells found may not represent the maximum number whose axones go to the periphery. Does the difference between operated and control sides of 468 cells indicate a loss in cells due to the operation? Probably not, because it can be accounted for on the basis of normal variation (one cat had only 71 cells on both sides, while another had 1247). In the normal human fetus, a greater number of cells are present on one side than on the other (Windle, '31). Furthermore, no sign of advanced cell

degeneration and no 'shadow cells' were encountered in any of the preparations. Therefore, this difference cannot be used to raise the total number of affected neurones.

No claim can be made that the course of all the efferent neurones in the ventral roots has been interrupted. Some of those which run in the gluteal, lateral femoral cutaneous, and inferior hemorrhoidal nerves; and all in the dorsal rami escaped injury. Had all these motor fibers been sectioned, the percentage of altered sensory cells might have been greater. However, there can be no question that the majority of the fibers in the ventral roots in question were sectioned when the genito-femoral, femoral, obturator, and sciatic nerves were cut. There is one other possibility in favor of the ventral root afferent pathway. The group of doubtful cells contains a few which may be affected by axone injury so slightly that they exhibit only one or two of the characteristics of chromatolysis. If these are added to the ones which are distinctly altered, the percentage of chromatolyzed cells will be increased still more. All that can reasonably be concluded is that ventral root afferent neurones may send a significant number of processes to a few of the peripheral nerves in some cats. Nevertheless, the failure of a great majority of the sensory cells to show chromatolysis must mean that most of them do not possess peripheral processes which run beyond the points at which the sections were performed.

Possibly these ventral root cells belong in the same category with those in the dorsal root ganglia which send 'recurrent' fibers into the ventral roots. Centripetal or 'recurrent' fibers have been demonstrated by myelin-degeneration methods (Dunn, '14; Sherrington, 1894; Windle, '31). Clark ('31) has found nerve fibers leaving the ventral as well as dorsal roots and coursing onto the pia mater in cats. There they terminate in sensory-like endings. Miss Dunn ('14) indicated that some centripetal ventral root fibers may pass into the substance of the spinal cord. The present experimental study cannot aid directly in determining the course of the processes of the ventral root cells. It is somewhat specu-

lative to suggest that their central fibers may reach the spinal cord by the dorsal roots and their peripheral ones pass to the meninges.

Other hypothetical arrangements, such as both processes passing over the dorsal roots or both over the ventral ones, might be considered. It could even be suggested that some of the cells are non-functional, possibly without long processes. Certainly, many of those observed in the normal ventral roots of the cats used in the present experiments were not strictly normal in appearance; vacuolization is a characteristic common to a large percentage of them.

CONCLUSIONS

The ventral spinal nerve roots in the lower lumbar and sacral regions of seven adult cats contain sensory type nerve cells varying in number from 275 to none at all.

Most of these neurones have no processes coursing in the nerves to the leg. Occasionally a small but significant number in some of the nerves undergo chromatolysis after section of the branches of the lumbosacral plexus.

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THE WEIGHT OF THE SKIN AND TELA SUBCUTANEA OF THE HUMAN FETUS¹

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THREE FIGURES

The present publication is a report on the weights of the skin and tela subcutanea and their relations to total body weight, total body length, and age during the fetal period.

Bischoff (1863) reported the weights of skin and subcutaneous tissue of three fetuses as part of a general study of the weight of parts and organs of the body at all ages. One newborn girl who had breathed a little had a skin weight of 337.25 grams, a tela subcutanea weight of 405.5 grams, and a total body weight of 2929 grams. One 6-month fetus had a body weight of 497 grams, a body length of 28 cm., and a skin weight (including the subcutaneous fat, lymph, and salivary glands) of 73.5 grams. A stillborn male infant had a body weight of 2400 grams, a body length of 49 cm., and a skin weight (including subcutaneous fat, lymph, and salivary glands) of 480 grams. In 1903, Brandt edited and published Welcker's extensive analysis of weights of the component parts of animal and human bodies. He gave the analysis made by Welcker in 1866 of a 3-month male human fetus which had a body weight of 12.513 grams and a skin weight of 1.05 grams. Jackson ('09) found that the weight of the skin and subcutaneous fat of a 6-month fetus was 13 per cent of the body weight. He showed that this value is in fair

¹ This report is part of the thesis submitted in partial fulfillment of the requirements for the degree of Ph.D. under the direction of Prof. R. E. Scammon and Dr. Edith Boyd.

agreement with the 15 per cent for Bischoff's fetus of the same age, is more than the 8 per cent found by Welcker for a 3-month fetus, and less than the 20 per cent found by Bischoff for a stillborn infant and the summary value of 19.7 per cent for newborns given by Vierordt ('06). In 1925, Jackson reported that the skin weighed 136, 275, 270, and 316 grams for four atrophic infants measuring 1800, 3204, 3580, and 5334 grams in total body weight and 48, 54, 55.6, and 62 cm. in total body length, respectively. The weight of the skin varied from 6 to 9 per cent of the body weight.

No other workers have reported the weight of the subcutaneous tissue in the fetal period, but two have weighed the body fat. Fehling (1877) reported that the weight of the chemical fat is from 0.6 to 9.1 per cent of the body weight in twenty fetuses ranging from 4 months of age to birth. Camerer ('02) found the chemical fat to be on an average 12.3 per cent of the total body weight at birth.

The few weights of skin and tela subcutanea given by these and other workers for postnatal life may be found either in Vierordt's ('06) tables or in Welcker and Brandt's ('03) summary.

The meager data reviewed indicates that the skin forms about 6 to 9 per cent of the total body weight during fetal life, while chemical fat, hence probably subcutaneous tissue, increases from a fraction of 1 per cent to approximately 10 per cent of the body weight.

In this study additional data on the weight of the skin and tela subcutanea have been obtained from twenty-eight fetuses ranging in length from 17 to 52 cm. These data were obtained as part of a total dissection dividing the body into skin, tela subcutanea, musculature, skeleton, and the remainder. The muscle and skeleton weights will be reported later.

In order to obtain representative fetuses for dissection, an attempt was made to use only those having a total body weight for total body length within ± 15 per cent of the calculated weight for length, according to the Scammon-

Calkins ('24 a) formula for total body weight and length.² Because of the slowness of the accumulation of these specimens, these conditions were not always met, so that the weights of three specimens were below and two above the set standard. All the weights, however, were well within three probable errors of calculated values. This indicates that these specimens are a reasonably good sample of fetuses.

Length was measured three times on a Schultz measuring board. The fetuses were weighed accurately on a balance scale to 0.1 gram. The skin was carefully removed from each fetus. Any fascia left on the skin was scraped off with the sharp edge of a knife. All hair and nails and the entire auricle were included with the skin weights. The tela subcutanea was dissected from the superficial muscles. The so-called sucking pad and all of the fat of the ischial rectal fossae were included with it. To prevent drying, each tissue was returned to 10 per cent formalin as soon as it was removed from the body. When ready to weigh, each tissue was dried between paper towels under approximately constant and even pressure of the fingers until no more moisture appeared on the paper. The tissue was then accurately weighed to 0.01 gram on an analytic balance. The dissection was done by several people, but, in order to keep the errors at a minimum, all the drying and weighing was done by myself.

The data for the twenty-eight specimens are summarized in table 1. The accuracy of these data is influenced to some degree by the effect of formalin as well as technical errors. The extraneous factors, however, are probably not sufficient to affect the general relations of the weights of the skin and tela subcutanea to body length and weight. Scammon and Calkins ('29) give a detailed analysis of the errors due to formalin and technical procedures.

When the weights of skin and tela subcutanea are plotted against body weight, the skin appears to have a rectilinear relation to total body weight, while subcutaneous tissue has

² $BW = (0.26 L)^{3.108} + 4.6$, where L is total body length in centimeters and BW is total body weight in grams.

a curvilinear (fig. 1). The rectilinear trend is the one usually found for the relation of the weights of organs and body parts (Scammon, '25, '26, '27 a and b, '30, and Wald and Scammon, '32) and may be expressed by the formula:

$$SW = 0.06447BW - 6.1173 \quad (1)$$

TABLE 1
Observed weights of skin and tela subcutanea in fetal life

FETAL AGE ¹	BODY LENGTH	BODY WEIGHT	SKIN WEIGHT	TELA SUBCUTANEA WEIGHT
<i>Lunar month</i>	<i>Cm.</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
4.2	17.4	100	2.8	0.6
4.5	19.0	156	9.0	...
4.9	22.3	246	8.0	0.5
5.1	23.2	250	12.6	1.8
5.1	23.7	287	10.5	0.7
5.4	25.6	334	17.1	2.6
5.4	25.7	329	17.3	2.0
5.5	25.9	442	20.6	2.7
5.6	26.7	533	22.9	1.5
5.8	28.1	442	17.6	3.3
5.8	28.2	437	16.7	...
6.0	29.2	451	17.6	3.3
6.1	29.6	570	33.5	10.9
6.4	31.4	548	38.6	12.6
6.4	31.5	706	40.7	28.8
6.7 ²	33.3	696	26.9	13.6
6.8	33.9	746	28.8	14.1
7.0	35.2	852	34.4	13.4
7.1	35.8	1053	81.1	50.0
7.2	36.4	943	55.1	19.8
7.2	36.4	947	60.2	40.6
7.4 ²	37.2	1089	67.9	
7.7	38.8	1234	83.2	123.5
8.2 ²	41.3	1530	68.1	87.3
8.3 ²	42.0	1444	102.4	110.4
8.6	43.5	1783	105.2	116.0
8.7	44.1	1898	140.2	164.2
10.4	52.2	3425	239.0	483.8

¹ Calculated from the Scammon-Calkins ('23, '29) age-length formula, $T = 2.3 + \frac{2.5L}{28} + \frac{L^2}{784}$, where T is age in lunar months and L is the crown-heel length in centimeters.

² Fresh specimens.

when SW is skin weight in grams and BW is total body weight in grams, and the numerical constants were determined by the method of averages from the observed weights. The calculated values have a mean absolute deviation of 11 grams and a mean relative deviation of 24 per cent from the observed weights.

According to this expression, the skin increases approximately 0.06 gram in weight for each gram of increase in total body weight.

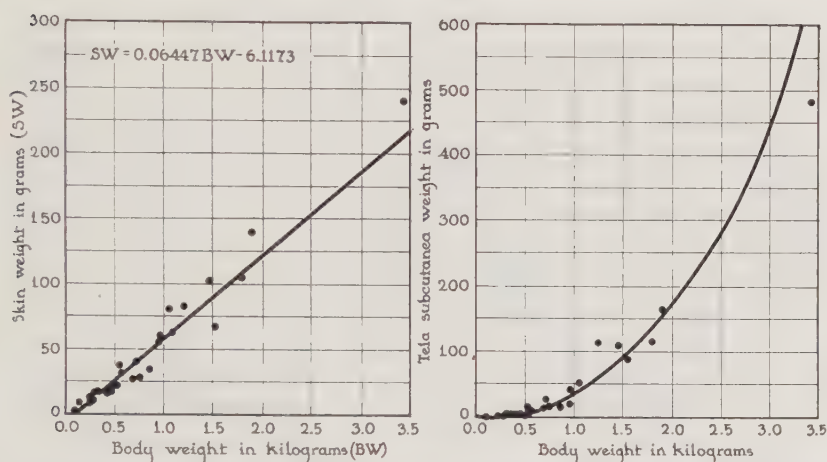


Fig. 1 Graphs illustrating the relations of the weight of the skin and tela subcutanea to the total body weight. The solid dots represent the individual observations. The solid line curve in the skin panel represents the empirical formula. In the tela subcutanea panel the solid line curve is drawn in by inspection.

The analysis of the curvilinear relation of tela subcutanea weight to body weight was limited to the graphic method of drawing in a curve by inspection after two applications of 3-point smoothing to the observed values. The estimated weights of the tela subcutanea read from the curve for 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kg. of body weight are 6.0, 38.5, 100.0, 176.0, 273.5, and 383.0 grams, respectively. According to these estimated values and the curve (fig. 1), as the body increases in weight from 0.5 to 3.0 kg. the subcutaneous tissue

increases slowly in amount until the body weighs about 1.5 kg., after which the subcutaneous tissue increases very rapidly.

When the weights of the skin and tela subcutanea are plotted against body length, both have curvilinear relations to body length, but the curves are strikingly different (fig. 2).

The curve for the weight of the skin and body length has the general form of an exponential curve expressed by the formula:

$$SW = aL^b, \quad (2)$$

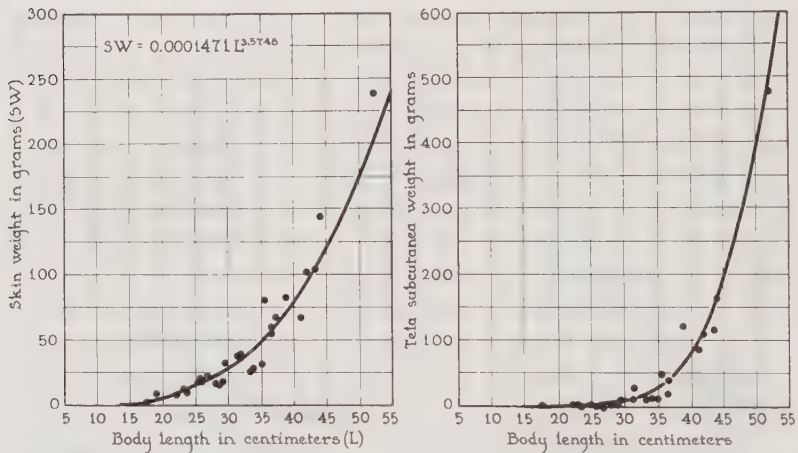


Fig. 2 Graphs illustrating the relations of the weight of the skin and tela subcutanea to the total body length. The solid dots represent the individual observations. The solid line curve in the skin panel represents the empirical formula. In the tela subcutanea panel the solid line curve is drawn in by inspection.

of which the logarithmic form is:

$$\log SW = \log a + b \cdot \log L. \quad (3)$$

When the constants $\log a$ and b are determined from the logarithms of the observed values by the method of averages, equation 3 becomes:

$$\log SW = -3.8324 + 3.5748 \log L \quad (4)$$

and equation 2 becomes:

$$SW = 0.0001471L^{3.5748}. \quad (5)$$

The mean absolute deviation of observed from calculated values is 9 grams, and the mean relative deviation is 22 per cent.

The analysis of the curvilinear relation³ of tela subcutanea weight to total body length was limited to drawing in a curve by inspection to the observed values after two applications of 3-point smoothing. The estimated values read from the curve for 15, 20, 25, 30, 35, 40, 45, and 50 cm. of body length are 0.5, 1.2, 2.5, 8.5, 29.0, 77.5, 197.0, and 402.0 grams, respectively.

TABLE 2

Calculated or estimated weights, velocities, and relative velocities of skin and tela subcutanea in fetal life

FETAL AGE	BODY LENGTH ¹	SKIN			TELA SUBCUTANEA		
		Calculated weight	Velocity	Relative velocity	Estimated weight	Velocity	Relative velocity
<i>Lunar month</i>	<i>Cm.</i>	<i>Grams</i>	<i>Grams per month</i>	<i>Per cent per month</i>	<i>Grams</i>	<i>Grams per month</i>	<i>Per cent per month</i>
5	22.9	10.7	11.3	105.7	2	0.7	33.9
6	29.2	25.6	19.1	74.6	6	12.2	203.5
7	35.1	49.3	28.0	56.8	30	40.3	134.3
8	40.6	82.6	37.8	45.8	85	83.1	97.8
9	45.7	126.4	48.1	38.0	220	209.5	95.2
10	50.5	180.9	58.8	32.5	422		

¹ From Scammon-Calkins fetal age-weight-length table ('24 b).

These relationships of skin and tela subcutanea weights to length were shifted to a time scale of lunar months, according to the lengths for each lunar month given in the Scammon-Calkins ('24 b) table. The corresponding values for skin weight (table 2) were calculated from equation 4, and those for tela subcutanea were read from the graph in figure 2. The resulting curves are shown as solid black lines in figure 3, along with the broken line curves obtained in the same manner from skin weight-body weight formula and tela subcutanea weight-body weight graph.

³ In work in progress, Scammon has found this type of curve is characteristic of the relation of the weight of fat and other chemical constituents of the body to total body length and that this curve may be represented by the equation of compound interest.

Both skin and tela subcutanea have a curvilinear relation to age, but, again, the tela subcutanea curve has a slower rise in the early fetal life and a more rapid rise in late fetal life than the curve for skin weight. This is more clearly illustrated by the curves of velocity and relative velocity

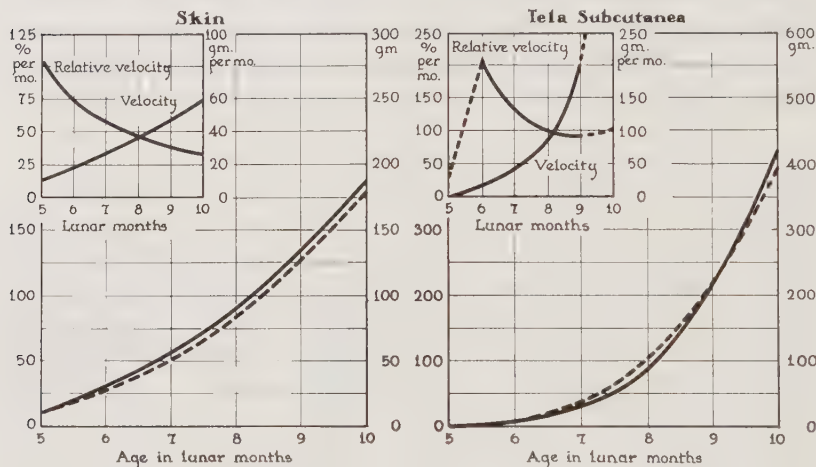


Fig. 3 Graphs illustrating the relations of the weights of the skin and tela subcutanea to age. The solid line in the skin panel represents the weights calculated from length according to body length for each month of age, and the broken line represents the weights calculated from body weight according to body weight for each month of age. The insert represents absolute and relative velocities calculated from the skin weight-body length formula shifted to the time scale. The solid line in the tela subcutanea panel represents the weights read from the curve for tela subcutanea weight and total body length according to total body length for each month of age, and the broken line represents weights read from tela subcutanea weight-body weight curve according to total body weight for each month of age. The insert represents the velocities and relative velocities read from the curve for tela subcutanea weight and age with a tangentometer. The dotted line indicates where the trend of the curve is questionable.

placed in the inserts. The velocities for skin weight were obtained algebraically from the first derivative of the skin weight-body length formula shifted to a time basis. The velocities for tela subcutanea were read from the age-tela subcutanea curve (fig. 3) by means of a tangentometer. The numerical values are given in table 2.

At 5 fetal months the skin weight is increasing at the rate of 11 grams per month, and by 10 fetal months at approximately 60 grams, or five times as fast at birth as in early fetal life. In contrast, the tela subcutanea weight is increasing at a slow rate of less than 1 gram per month at 5 fetal months, and at a very rapid rate of 210 grams by 9 fetal months (the value for 10 fetal months could not be read from the curve), or approximately 300 times as fast at 9 lunar months as in early fetal life. The relative rate of growth of the skin decreases rapidly from 106 per cent per month at 5 fetal months to 46 per cent at 8 fetal months and then slowly to 33 per cent per month at birth. The relative rate of growth of the tela subcutanea increases abruptly from 34 per cent per month at 5 fetal months to 203 per cent at 6 fetal months, then decreases gradually to 95 per cent per month at birth. This curious relative rate may be due to artifacts introduced by the few cases and graphic methods used.

Certain general relations of the weights of skin and tela subcutanea are brought out by a series of indices for each fetal month based on the calculated values of their weights and the total body weight (table 3). The skin forms 4 per cent of total body weight at 5 fetal months and 6 per cent at birth, while tela subcutanea forms only about 1 per cent at 5 fetal months and 13 per cent, or twice as much as the skin, by birth. These results are in agreement with those of earlier workers for skin weight and chemical fat weight, respectively, quoted above.

The skin weight-tela subcutanea weight ratio shows that the skin weighs approximately five times as much as the tela subcutanea at 5 fetal months, while by birth the subcutaneous tissue weighs twice as much as the skin. Also, at 5 fetal months the skin has reached approximately one-twentieth of its birth weight, while the tela subcutanea is only one-two hundredth of its birth weight. By 8 fetal months the skin has one-half of its birth weight and the tela subcutanea has only one-fourth of its birth weight. These relations redemonstrate the strikingly slow rate of increase in

subcutaneous tissue in the early fetal months and its very rapid increase in the last 2 fetal months.

In summary, the skin weight has the same general growth characteristics of other organs and parts of the body, having a rectilinear relation to body weight, curvilinear relations to body length, for which empirical formulas have been deter-

TABLE 3

Indices calculated from either the calculated or estimated weights of skin, tela subcutanea, and total body for each lunar month of fetal life

FETAL AGE	BODY WEIGHT ¹	CALCULATED SKIN WEIGHT ² (SW)	ESTIMATED TELA SUB- CUTANEA WEIGHT ³ (TW)	SW TW x 100	PERCENTAGE RATIO OF SKIN WEIGHT TO:		PERCENTAGE RATIO OF TELA SUBCUTANEA WEIGHT TO:	
					Total body weight	Its birth weight	Total body weight	Its birth weight
<i>Lunar month</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>					
5	261	10.7	2.0	535.0	4.1	5.7	0.8	0.5
6	552	29.5	6.5	453.8	5.3	15.8	1.2	1.7
7	971	56.5	35.0	161.4	5.8	30.2	3.6	8.9
8	1519	91.8	99.0	92.7	6.0	49.0	6.5	25.3
9	2196	135.5	215.0	63.0	6.2	72.4	9.8	54.8
10	2999	187.2	392.0	47.8	6.2	100.0	13.1	100.0

¹ From Scammon-Calkins fetal age-weight-length table ('24 b).

² Calculated from body weight according to equation 1.

³ Read from curve in second panel of figure 1 according to body weight for each lunar month.

mined. The weight of the tela subcutanea forms an exception to this general pattern of growth, since its relation to body weight is curvilinear, for which no empirical formula has been found. Its curvilinear relation to body length⁴ determined graphically shows a slower rate of growth than other structures in the early fetal months and a strikingly fast rate in the last month and a half.

⁴ See footnote 3 on page 133.

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THE INCIDENCE OF TUBULAR GLANDS AND CONCRETIONS IN THE ADULT HUMAN HYPOPHYSIS CEREBRI¹

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ONE PLATE (FOUR FIGURES)

The polymorphic and rudimentary character of the pars intermedia of the human hypophysis has received considerable attention in recent years (for literature see Rasmussen, '28, and Bucy, '32). In the majority of adults the parenchyma represents less than 5 mg. of tissue or a little more than 1 per cent of the total epithelial portion of the gland. A similar condition exists in the chimpanzee and orang-utan (Plant, '22, '30). In lower animals there are noticeable differences in the general histology and the relative proportion of this lobe. In the latter connection, Wislocki ('29) reports an entire absence of this region in the porpoise, as is also frequently the case in birds, in contrast to a fair proportion, varying from about 5 or 6 per cent (woodchuck, rat, and some urodeles) to 25 per cent (frogs) of the total epithelial portion of the organ.

In the hypophysis of man and man-like apes, the intermediate region may be represented by a thin, imperfect sheet of cells, frequently only a single layer of cells in thickness in places. In many human cases there are, in addition, a variable number of basophilic cells that may be scattered for some distance into the posterior lobe and be unrelated to colloid (Rasmussen, '30). There are usually also colloid

¹ Aided by a grant from the research fund of the Graduate School, University of Minnesota, and from the Grant-in-Aid Fund of the National Research Council.

vesicles, consisting of a single layer of cells presenting a variety of forms and staining reactions, including ciliated and mucous secreting cells (Rasmussen, '29). Since follicles, both with and without colloid, are quite regularly found in the upper regions of the gland near the attachment of the stalk to the posterior lobe, Pietsch ('30) recognizes a cystic region. A transitional zone between pars tuberalis and this cystic portion is divided by Pietsch into a nuclear region, next to the cystic, and a transitional zone proper, which more or less surrounds the lower portion of the infundibulum and is continuous with pars tuberalis. The cells of these different subdivisions differ sufficiently cytologically to suggest to Pietsch that they serve different functions.

GLANDS

On account of the uncertainty regarding the secretory function of pars intermedia, especially in man and higher animals, the existence of simple or branched tubular glands extending backward into the posterior lobe, but continuous with pars intermedia, is of special interest. The reconstructions of these glands by Lewis and Lee ('27) constitute the best representation of their morphology. As shown in figure 1, they may present the general features of the end-pieces of salivary glands even to the possession of intercalated ducts. They vary, however, from such compound glands to simple short tubules.

In most cases they are inconspicuous and confined to the region of pars intermedia, extending posteriorly very obliquely or more or less parallel to the residual lumen, with a tendency to crowd in between the pia and the posterior lobe along the lateral margins of the gland. However, they may penetrate into the posterior lobe anywhere and from any part of the pars intermedia.

It is generally stated that these glands are most prevalent during the first four years of life, but no adequate quantitative data exist to check this impression.

When we came to cutting adult human female hypophyses, it appeared that these gland-like tubules were encountered oftener than in the adult male organ studied previously; but since the work was spread out over a period of 10 years, such general impressions might be due to the fact that we were more interested in these glands by the time we came to sectioning female material. In order to see if there is an actual sex difference, a careful systematic examination was made to every twentieth section of complete 10 μ series of the normal hypophysis of 111 human adult males from 20 to 76 years of age, and of 124 females, 19 to 84 years. The results are tabulated in table 1.

TABLE 1

Absolute and relative number of normal adult human hypophyses containing tubular gland in pars intermedia or projecting into posterior lobe

NUMBER OF CASES	SEX	AGE	REMARKS	NUMBER CONTAINING GLANDS	PER CENT
47	Male	Over 50 years		23	49
64	Male	Under 50 years		31	48
111	Male	20 to 76 years	Total	54	49
36	Female	Over 50 years		25	69
64	Female	Under 50 years	Non-pregnant	52	81
24	Female	Under 50 years	Pregnant	20	83
124	Female	19 to 84 years	Total	97	78

The criteria used to distinguish these glands from vesicles, many of which are mere dilatations of these gland-like tubes, were, *a*) the columnar or slightly pyramidal shape of the cells; *b*) small lumen; and, *c*) character of the cytoplasm.

It will be seen that these glands were identified in a much larger percentage of females than males, being found in about 49 per cent of males, as compared with 78 per cent in females, with no age differences in the male. The apparently higher percentage (81 per cent) of glands in non-pregnant women under 50 years of age, as compared with 69 per cent in females over 50 years, is not statistically significant, for when tested by computing the Chi-square value for a fourfold table (Dunn, '29), it is found that there are 18 chances in 100

that this age difference is merely due to random sampling. For most purposes, in order to be considered significant, there should not be over 5 chances in 100 that the difference is due to sampling.

Regardless of the supposed great increase in activity of the hypophysis during pregnancy, there appears to be no significant difference in the number or prominence of these glands during and immediately subsequent to pregnancy.

Although only about forty uniformly spaced sections from each gland were systematically examined, the size, irregular course, and multiple character of these glands make it very unlikely that they have been overlooked in a significant number of cases.

Not enough cytology has been done on properly fixed material to warrant any speculation on the amount or nature of the secretion, if any, elaborated by these tubules.

CONCRETIONS

It was noted that the microtome knife became nicked oftener while cutting female hypophyses. In sections of the infundibulum it has been noted that there were calcified concretions in some of them. Similar structures were also seen occasionally in the pia covering the main part of the gland (fig. 2). It was therefore of interest to know if there were any sex differences in regard to the incidence of these concretions. The same series of sections were therefore checked for the presence of concretions simultaneously with the exploration for glands.

The results, as recorded in table 2, show that they are present in almost twice as many women as in man, with no age differences in either the male or female.

When present, these concretions are almost invariably located in the pia on the upper aspect of the gland near the attachment of the stalk (fig. 2) and within and along the stalk. The surrounding connective tissue usually forms a definite wall about them, as shown in figure 4. They have occasionally, though rarely, been found inside of a blood vessel, as is

certainly the case illustrated in figure 3, which is from a section passing obliquely through the infundibulum some distance above the main lobes of the hypophysis.

From their morphology and staining reaction they appear to be similar to the 'brain sand' (acervulus cerebri) found in the pineal gland. The degree of calcification varies considerably, and, as shown in figure 4, the staining reaction suggests differences in the composition in different concentric strata of the same concretion. According to the summary by del Rio-Hortega ('32), the mineral matter is largely carbonates and phosphates of calcium, potassium, and magnesium deposited in an organic substratum.

TABLE 2

Absolute and relative number of normal adult human hypophyses containing concretions in the pial investment

NUMBER OF CASES	SEX	AGE	REMARKS	NUMBER CONTAINING CONCRETIONS	PER CENT
47	Male	Over 50 years		17	36
64	Male	Under 50 years		24	37
111	Male	20 to 76 years	Total	41	37
36	Female	Over 50 years		26	72
64	Female	Under 50 years	Non-pregnant	46	72
24	Female	Under 50 years	Pregnant	16	67
124	Female	19 to 84 years	Total	88	71

In the pia, chorioid plexus, and psammoma, such concretions evidently arise in connective tissue and may be an expression of the tendency for calcification to occur in hyalinized connective tissue. One might, therefore, expect a higher incidence of such concretions in and about the hypophysis, as well as other places, in older individuals. As far as the region of the hypophysis is concerned, this is apparently not the case. For a summary of the general subject of pathological calcification see Barr ('32).

In the pineal gland it appears that the concretions may arise from the neuroglia elements by the deposition of calcium salts starting in the expanded part of a process, the cell ultimately disintegrating and freeing the calcareous mass (del Rio-Hortega, '32; Cooper, '32).

OTHER SEX DIFFERENCES IN THE HYPOPHYSIS

Besides a higher incidence of tubular glands and concretions in the human female hypophysis, some other sex differences have been noted in various animals. The anterior lobe of the female is larger in the rat, rabbit, and cattle. In the woodchuck the reverse seems to be the case. In this form the pars intermedia is also larger in the male (Rasmussen, '21).

THE QUESTION OF DIRECT COMMUNICATION BETWEEN BLOOD SINUSES AND RESIDUAL LUMEN

Incidentally, we have seen nothing in our examination of sections of nearly 250 normal human hypophyses (including several from children) to support the recent announcement by Brander ('32) to the effect that he could apparently establish a direct communication between the lower pole of the residual lumen and neighboring blood sinuses (intercavernous sinus). The tissue between the connective tissue capsule of the gland and the residual lumen may be very thin at the lateral and inferior margins of the intermediate region, especially if the lumen is greatly distended with colloid. In manipulation of the gland, both before and after fixation, and in spreading the sections after being cut, there is a natural tendency for the anterior lobe to become partly detached from the rest of the organ, so that breaks in the thinnest part of the walls of the lumen frequently occur. If an actual communication, as described by Bander, exists so that relatively large masses of colloid could get from the residual lumen directly into the blood sinuses, one wonders why the residual lumen is not regularly filled with blood. Masses of colloid much larger than blood corpuscles have unquestionably been found in the blood sinuses (Rasmussen, '27), but it is not clear how they get into the blood stream. It is more likely that a more diffusible form of the colloid passes through the endothelial wall and other intervening tissues and that larger aggregates are formed in the lumen of the vascular sinusoids and thus ultimately get into the venous sinuses.

SUMMARY

1. As a result of a systematic exploration of every twentieth section of complete 10 μ series of 235 carefully selected normal adult human hypophyses (111 males, 20 to 76 years of age, and 124 females, 19 to 84 years of age) tubular gland-like structures extending posteriorly from the residual lumen and frequently into the posterior lobe, were found in about 49 per cent of the males and 78 per cent of the females.

2. While only 69 per cent of the 36 females over 50 years of age contained such glands, the difference is not sufficient, considering the number of cases involved, to constitute statistical proof of a decrease in those over 50 years of age, there being 18 chances in 100 that the difference is merely due to random sampling.

3. There is no significant difference in the incidence of glands in 24 pregnant females as compared with 64 non-pregnant females.

4. More or less calcified concretions, resembling acervuli (brain sand) of the pineal body, were present in the pial investment of 37 per cent of the males and 71 per cent of the females.

5. In neither sex was there any difference in the incidence of concretion in those over 50 years of age and those under 50 years of age, nor in pregnant women.

6. The concretions are largely confined to the pia on the upper aspect of the hypophysis and along the infundibulum. They may occur in pars tuberalis.

7. Other sex differences observed in the hypophysis of animals are mentioned, the most commonly noted being a larger anterior lobe in the females of several species, although the reverse may occur.

8. No support was found for the statement that there is a patent communication between the residual lumen and neighboring venous blood sinuses permitting masses of colloid to pass directly from the residual lumen into the blood stream.

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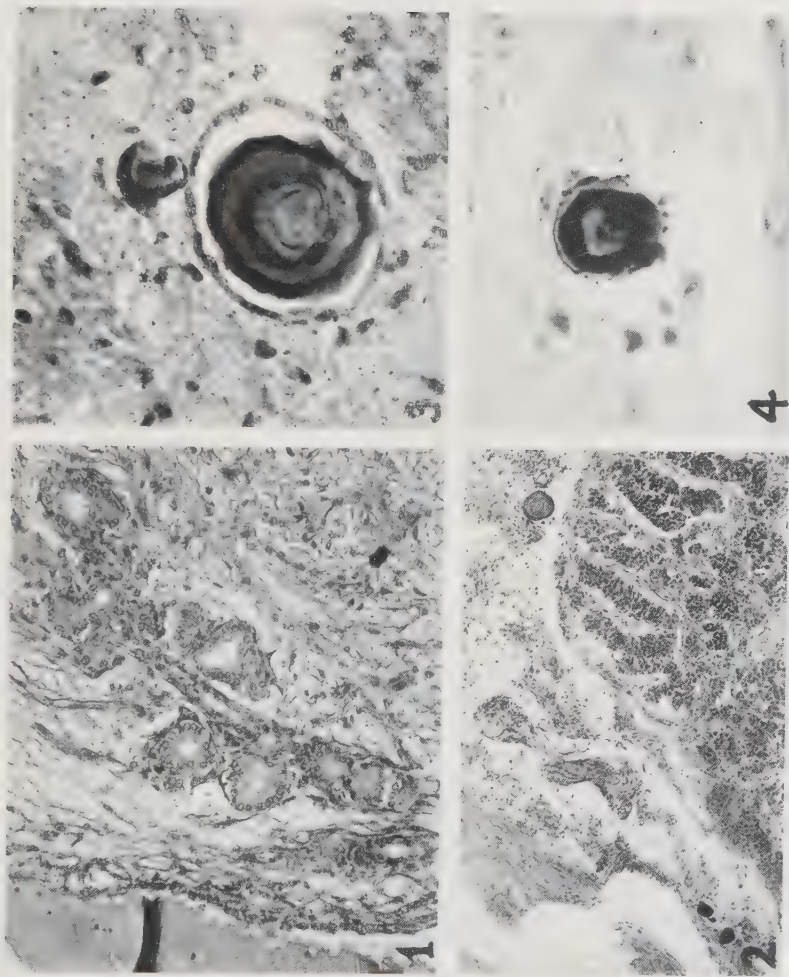
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PLATE

PLATE 1

EXPLANATION OF FIGURES

- 1 Photomicrograph of gland-like projections from pars intermedia in posterior lobe of the hypophysis of a normal 25-year-old human female. Note a general resemblance to the end piece of a typical tubulo-alveolar gland with intercalated duct. Colloid in the residual lumen and pars intermedia are to the left. Formalin fixation, 10 μ sections, hematoxylin and eosin stain.
- 2 Photomicrograph of three concretions in the pia in the angle between the anterior lobe (below and to the right) and upper pole of the posterior lobe (above and outside the limits of the photograph). Typical concentric lamination is evident in the largest one (at the right). Two smaller ones are seen in the lower left-hand corner. From hypophysis of another normal adult human female, 25 years old. Same technique as preceding.
- 3 Photomicrograph of concretions from the infundibulum of a normal adult human male hypophysis showing a concretion within a blood vessel and a smaller one just outside. Same technique as preceding.
- 4 Photomicrograph of a typical concretion from the pia of a normal adult human hypophysis from a female 64 years old, showing a very dark cortical zone and a lighter medullary region, undoubtedly reflecting a higher degree of calcification in the cortical layer. The tendency for the formation of a connective tissue capsule about each concretion is also illustrated. Same technique as preceding.



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PROCEEDINGS OF THE THIRTIETH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

ATLANTIC CITY, N. J., DECEMBER 28-31, 1932

PROGRAM

(All sessions were held in the Municipal Auditorium)

In addition to the 213 papers published in the November supplement of this journal, the following eight titles were added by vote of the Society.

Read

214. THE RELATION OF TASTE BUDS TO HUMAN CIRCUMVALLATE PAPILLAE. L. B. Arey, Northwestern University. (No abstract received.)

215. PIGMENT GENERATION IN REGENERATING HAIR OF THE MOUSE. Katherine S. Foote, Louisiana State University. (Introduced by E. H. Behre.)

There are extrinsic as well as intrinsic influences which will affect the generation of pigment in regenerating hair of the mouse, as found by scientific experimentation with mice of determined genotypes. Only immediate change with nature of change in the coloration of hair and location of pigment were considered in the experiments. Microscopic examinations of hair from control and experimental groups were made, and a histological basis for comparison of intensity and location of pigment was determined. Detailed records point to the following judgments:

Light (and darkness) has an effect upon pigment formation, although it is not consistent and is combined with other undetermined factors.

Local hyperaemia produced by xylol causes an inhibition of pigment formation in some hairs.

Applications of resorcinol produce a darkening of coat color and increase pigment formation.

Electric shock does not affect pigment formation in mice.

Adrenalin does not affect pigment formation in mice.

Pituitrin, if given in sufficient quantities, will produce a slight darkening of coat color and an increase in pigment formation.

Further investigation is under way concerning formations closely resembling nuclei found through the microscopic examination of hair of mice treated with xylol.

216. THE EFFECT OF SUBCUTANEOUS INJECTIONS OF LARGE AMOUNTS OF COD LIVER OIL ON THE THYROID GLAND OF THE WHITE RAT. T. C. Sherwood, Katherine Carr, Louis A. Toth, University of Kentucky. (Introduced by the Secretary of the Society.)

Results obtained on fifty-seven adult white rats which were injected subcutaneously with large amounts of cod liver oil showed no loss in weight. However, upon studying the thyroid glands, a decided change had occurred, namely, the absence of colloidal material as compared with control animals and a decided increase in size of the acinar cells.

Studies on the basal metabolism of these animals showed no change in caloric output, as might have been expected with so decided a change in the thyroid gland.

217. STUDIES IN DIURNAL RHYTHM NO. 1. Hugh H. Darby, Bartol Research Corporation. (Introduced by the Secretary of Section F.)

The emergence of pupae from the puparium of *Anastrepha ludens* (Loew) showed a marked diurnal rhythm. The emergence of flies started about 4.00 A.M. and reached the peak between the hours of 9 to 11 A.M. A steady decline followed. Attempts were made to alter the rhythm with temperature, mechanical vibration, and light. These are reported in detail. Other methods of disturbing the rhythm are also reported.

By title

218. CONTRIBUTION TO THE QUESTION OF CHROMATIN DIMINUTION. A. F. Huettnner, E. Spotkov, and H. Wald, New York University.

The process called chromatin diminution occurs at three different periods in the life cycle of the organism. The first type is found in *Ascaris megalocephala* from the second to the fifth cleavages and in the fly *Miastor* in the third and fourth cleavages only. In *Dytiscus*, an example of the second type, diminution occurs in oogenesis, during which a differentiation takes place in the oogonial nucleus. In the third type of diminution, as exemplified by the *Lepidoptera*, the process takes place after the nuclear membrane has been broken down, namely, in the anaphase of the first maturation division.

That the eliminated substance is chromatin has been demonstrated by many investigators using Heidenhain's and Delafield's hematoxylin. Recently Bauer ('32), with the use of the Feulgen reaction, ascertained the chromatic nature of the eliminated material in *Ascaris* and *Dytiscus* and its non-chromatic nature in *Ephestia*.

Evidence that the material is morphologically associated with the chromosomes, as described by Fogg in 1930, is open to doubt. Work on *Drosophila* and *Ephestia* indicates that there is no sound basis for assuming such a relationship.

An achromatic origin for the presence of what has been termed elimination substance in the equatorial plane of the anaphase of the first maturation division in *Ephestia* is suggested. This is made manifest by the similarity of this structure in *Ephestia* to the mid-body in *Drosophila* which is formed from the condensation of the spindle fibers, probably together with other cytoplasmic material in the anaphase of the first mitotic division.

219. CONTROL OF POLARITY IN THE ALGA *GRIFFITHSIA* BY ELECTRIC CURRENT. Victor Schechter, Columbia University. (Introduced by W. H. Cole.)

If the filamentous red alga, *Griffithsia bornetiana*, is collected and placed in dishes of sea water in the laboratory, many cells in the basal and medial regions will form rhizoids within 2 or 3 days. The object of the experiments was to determine the effect of direct electric current on rhizoid formation. A single clump, in each experiment, was separated into twelve or more fragments of 100 or more cells each. The fragments were oriented alternately in dishes of running sea water so that they lay with and against the direction of the current, but always parallel to its path. Light and temperature were controlled and precautions were taken to avoid the effects of electrolytic products. In 20 out of 26 groups (each consisting of four fragments) there was definite effect in that the rhizoids arose from that end of the cells which lay toward the anode regardless of the original polarity. Shoots, which were observed only occasionally under the conditions of the experiment, usually originated opposite to the rhizoids. Thus complete reversal of polarity was indicated. Treated plants when removed from the experimental dishes were in as good condition as the controls. Data were also obtained on pH changes and electrophoretic migration of materials.

220. GROSS ASSIMILATION OF YOLK AND ALBUMEN IN THE DEVELOPMENT OF THE EGG OF *GALLUS DOMESTICUS*. Alexis L. Romanoff and Anastasia J. Romanoff, Cornell University. (Introduced by the Secretary of the Society.)

By the exact quantitative gross determination of yolk and albumen (wet and dry) on a large number of fertilized fowls' eggs it is concluded that:

The yolk and albumen of the fowl's egg undergo most profound changes in the course of embryonic development. Most of these changes involve transference of water from one part of the egg into another.

The water content of albumen rapidly decreases during the first part of incubation. At the expense of this water there is a noticeable swelling in the yolk; a large portion of water goes for the formation of embryonic membranes, some indirectly for the growth of embryo, and finally some of it is lost through external evaporation.

As a result of these modifications the yolk at first gains in its moisture content, then begins to decrease, and after two weeks of incubation reaches its original value.

The dry substance of albumen, irrespective of its water content, is almost exclusively assimilated during the second half of incubation, until it completely disappears just before hatching.

The dry substance of yolk is gradually being assimilated by the developing embryo from the beginning of incubation, and the largest portion of it is used in the few days immediately before hatching. A considerable part of the yolk is used after the bird's exclusion from the shell.

The decrease of dry weight of yolk and albumen combined corresponds to the amount of katabolized egg substances, and it is directly proportional to the progressive development of the embryo.

221. THE DEVELOPMENT OF THE PLACENTA AND FETAL MEMBRANES OF THE THIRTEEN-STRIPED SPERMOPHILE (*CITELLUS 13-LINEATUS*, MITCHILL). H. W. Mossman and Louis A. Weisfeldt, University of Wisconsin. (Introduced by the Secretary of the Society.)

The complete morphogenesis of the fetal membranes of this ground squirrel has been worked out. The beautiful collection of early stages made by the late Prof. Thomas G. Lee, of the University of Minnesota, has kindly been put at our disposal, and has been of great value. As described by Lee, implantation is at first on the antimesometral side. Later, this connection disappears and a true yolk-sac placenta (ectoplacenta) is established on the sides (periplacental portion) of the gestation chamber. This disappears with the development of the allantoic placenta on the mesometral side. Allantoic vascularization of the placenta first begins at the 7-mm. stage. Inversion of germ layers occurs late as in the rabbit. The amnion forms by folding. The placenta is discoidal, labyrinthine, and of the hemochorialis type. After midterm, decidual cells are scarce. The trophoblastic tubules are unusually large, averaging about 50μ in diameter to 10μ for the fetal capillaries which form a network around each tubule. This is of interest from the standpoint of the physiology of interchange between the two blood streams. Völker, studying the European spermophile, thought that the placental syncytium arose from the syncytium formed by the degenerating uterine gland epithelium. We have shown that instead it is a true plasmodi-trophoblast.

Twelve papers scheduled to be read were omitted from the program, due to the absence of the authors. The demonstrations were not as satisfactory as they should have been, due to the crowded condition of the room. It is planned next year to improve demonstration facilities so that there will be ample room and sufficient equipment. Attendance at the sessions for reading of papers was approximately as follows: Wednesday morning, Physiology 70, Embryology 80; Thursday morning, Physiology 130, Cytology 100; Thursday afternoon, Physiology 150, Miscellaneous, 25; Friday morning symposium, 225.

On Wednesday evening in The Ambassador the Biologists' Smoker was attended by about 500 biologists.

On Thursday evening, the Zoölogists' Dinner, in cooperation with Section F, was held in the Venetian Room of The Ambassador, with 179 persons in attendance. The dinner address was given by the retiring Vice-President of Section F, Charles Zeleny, on 'Genetics and Embryology.'

On Friday morning a very successful symposium was held on the subject, 'Embryonic Determination' with 225 in attendance. Invitational papers by E. G. Conklin, R. G. Harrison, D. H. Tennent, and P. Weiss were read and discussed. B. H. Willier was unable to be present to read his paper.

BUSINESS MEETING

At the business meeting on Thursday afternoon, December 29th, Vice-President L. V. Heilbrunn presided in the absence of W. C. Curtis. About sixty-five were in attendance. The minutes of the 1931 meeting as published in the January, 1932, *Anatomical Record* were approved. The following reports were accepted and ordered spread on the minutes.

Report of the Secretary

During the year 1932, 13 members were dropped for non-payment of dues; 20 members (15 active and 5 associate) resigned; 7 names carried on the lists in error were dropped, and 2 active members died, leaving a membership of 589 on December 29, 1932. Later in the meeting, the election of 15 new active members, of 8 associates to active membership, and of 29 new associate members brought the total membership to 633, classified as follows: 5 life members, 502 active, and 126 associate. William Patten and George T. Lee died during the year and the following minutes were adopted and ordered sent to the respective families.

WILLIAM PATTEN

When Dr. William Patten died suddenly at Hanover, New Hampshire, on October 27, 1932, there passed away one of the most talented, resourceful, and original workers which this or any other society has known. To describe his powers and accomplishments adequately one would need a long array of adjectives. To break new paths in the venerable forest of accepted ideas, indomitable industry and patience are not enough; there are needed also courage and an unconquerable will, a rugged physique which can take punishment with equanimity, technological skill of the first order, and the visualizing powers of the born artist. All of these qualities were present and developed to such a degree in William Patten that all component and candid persons, whether they accept his original ideas and bold conclusions or not, must agree that he has enriched his favorite science with a priceless fund of well-attested facts that neither envy nor the tooth of time can ever gainsay or destroy.

Educated at Harvard and Leipzig, working in laboratories at Trieste, Naples, Milwaukee, Woods Hole, as well as in those of his own making, particularly at Dartmouth College, where 39 years of his active life were spent (1893–1932), he began a series of publications of solid excellence, remarkable alike for their illustrations, for he adorned whatever he touched, and for the underlying motive discernible in nearly all of them.

Professor Patten's first important papers on the embryology of insects and mollusks and on the eyes of mollusks and arthropods (1884–1889) were followed by those remarkable studies upon the development and nervous system of *Limulus* (1889–1900), which put in the shade about everything which had been previously written on that ancient and classical form. From 1900 onward his published work was mainly devoted to his now famous researches upon the Ostracoderms, and to the publication of his book on "The origin of vertebrates and their kin" (1912).

In the thirty-two titles of Doctor Patten's published works, as given in the bibliography prepared by his colleague, Professor Gerould, there is not a trivial number, and many represent the labor of years.

Practically all the zoölogical work which William Patten ever accomplished was brought to bear upon his favorite theory. He was assured that in the giant sea scorpions of the Silurian Age, and in the strange ostracoderms, their contemporaries, he had discovered the long-lost golden key to unlock the mystery of vertebrate descent. Upon finding that the precious ostracoderm fossils in museums were untouchable, he ransacked the earth for new materials which he could study at will. A treasure trove of *Bothriolepis* rewarded him in New Brunswick, and another of *Tremataspis*, a small and delicate ostracoderm, for which he had hunted in vain for over 30 years, at Esthonian Oesel, in the Baltic. Another small member of this group, found also at Oesel, and representing a new family and genus, was named with justifiable pride *Dartmuthia gemmifera*.

To William Patten comparative morphology and phylogeny were the fountain sources of all our knowledge of evolution; so the phylogeny of vertebrates presented to his inquiring mind the most important problems of the biologist; and "upon their solution," he thought, "depended our conception of the way in which evolution has actually taken place."

A most genial companion, "full of wise saws and modern instances," one who had traveled in the interests of science to the ends of the earth, artist, and athlete, with a brow and chin that betokened the thinker and the tenacity with which he held the truth as he saw it, William Patten will be sadly missed, but his works will follow him.

FRANCIS H. HERRICK.

THOMAS GEORGE LEE

Prof. Thomas G. Lee died on September 1, 1932, from injuries received in an automobile accident a few days previously. He had resided at Babson Park, Florida, since his retirement from the University of Minnesota as professor emeritus in 1929.

He was born at Jacksonville, New York, in 1860. He attended the University of Pennsylvania, where he received the degrees of B.S. and M.D. (1886). He was also awarded the degree of B.S. at Harvard University. During the last

two years at Pennsylvania he served as student assistant to Joseph Leidy in histology and embryology. Then he went to Yale University, where for 5 years he was lecturer in histology and embryology and director of the laboratory. He also taught these subjects for 1 year at Radcliffe College.

In 1892, he was called to the University of Minnesota as an instructor in the recently established school of medicine, where he taught embryology, bacteriology, and histology. In 1908, he was made professor and head of the department of anatomy. In 1913, he became professor of comparative anatomy.

Although quiet and modest in demeanor, Doctor Lee was firm and aggressive in his persistent efforts to increase the facilities and improve the standards of scientific laboratory work. As librarian, he also devoted much time to the foundation and development of the medical library.

He was active in the investigation of mammalian embryology, and published several important papers on the early development, implantation, and placentation of *Spermophilus tridecemlineatus* and related rodents. His collections of material in this field are extensive and valuable.

He was a member of the American Association for the Advancement of Science, Sigma Xi, American Society of Naturalists, American Society of Zoölogists (secretary-treasurer of the Central Branch from 1906 to 1908), American Medical Association, Minnesota Academy of Medicine, American Association of Anatomists, and Anatomische Gesellschaft.

He was highly esteemed by his colleagues and is affectionately remembered by thousands of former students, who speak of him familiarly as 'Tommy.'

C. M. JACKSON.

Report of the Representative of the Society on the Executive Committee of the Commission for the Standardization of Biological Stains

To the members of the American Society of Zoölogists:

As your representative on the Commission for the Standardization of Biological Stains, I wish to report the following:

During the year I contributed to the testing work of the Commission by giving my opinion on various samples of carmine, sudan III, and Congo red.

I also attended the annual meeting of the Executive Committee in New York on March 26th.

Owing to the resignation of Mr. French, a new vice-chairman was elected and such election fell upon the representative of your Society.

At this meeting we learned of the death of W. C. Holmes, long a faithful member of the Commission, who had contributed much on the chemistry and solubility of dyes and to whom we all owe a great debt of gratitude for his faithful work, given without remuneration, to the cause of Science.

At this meeting it was also decided to enlarge the scope of our journal, *Stain Technology*, to include microscopical technique and to indicate this in the title of the journal. The editor is, therefore, willing to accept original contributions to any phase of micro-technique, including work in sectioning and photography as well as in staining methods. Members of the Society who contemplate the publication of such papers can find a ready medium in this journal.

Respectfully submitted,

S. I. KORNHAUSER.

*Report of the Treasurer**Credits:*

Balance on hand December 17, 1931, when the accounts were last audited	\$2316.87	
Receipts from dues, 1932	2305.00	
Interest on deposits	29.92	
	\$4651.79	\$4651.79

Expenditures:

Secretary's expenses:

Traveling and incidentals at annual meeting	\$161.48	
Typewriter	85.05	
Stenographer	114.55	
Stationery, printing, supplies	110.05	
Postage and telegrams	45.05	
	\$516.18	\$516.18

President's expenses:

Letter ballot on dues	\$4.31	4.31
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Treasurer's expenses:

Printing and stationery	\$12.43	
Postage and supplies	25.18	
Stenographer	34.80	
Exchange and check tax	1.83	
Refunded dues	5.00	
	\$79.24	79.24

Biological Abstracts (by appropriation) ...	\$500.00	500.00
Union of American Biological Society (by appropriation)	200.00	200.00
Biological Abstracts (by assessment)	1215.00	1215.00
Deficit on Dinner at New Orleans	15.45	15.45
The Wistar Institute	348.32	348.32
Committee on Revision of Constitution (D. E. Minnich)	40.72	40.72

	\$2919.22	2919.22
		\$1732.57

Balance on hand December 23, 1932, deposited in the name of the American Society of Zoölogists, as follows:

Commercial Department, Cambridge Trust Company	349.35
Savings Department, Cambridge Trust Company	379.47
Cambridge Savings Bank	1003.75

\$1732.57

Decrease in the net funds of the Society since the report of last year, \$583.40.

Respectfully submitted,

ALDEN B. DAWSON, *Treasurer*.

Report of Auditing Committee

This committee, consisting of E. F. Adolph and C. L. Parmenter, reported that the accounts of the treasurer had been examined and found correct.

Report of Committee on Constitution and By-laws

The constitution as rewritten by the committee (D. E. Minnich, C. Grave, and W. C. Allee) and distributed to all members in September, 1932, was modified in a few places, upon recommendation of the Executive Committee, and adopted. Perhaps the most important changes were the limitation of associate membership to those who have had training in zoölogy equivalent to that required for the doctorate degree, and the opening of the program to associates without introduction. The new constitution and by-laws as adopted are printed in full herewith (pp. 167-170).

Report of the Committee on the Preservation of the Records of the Society

The committee appointed by President Curtis to examine and make permanent disposition of certain older records of the American Society of Zoölogists which have been temporarily stored in the Marine Biological Laboratory at Woods Hole, Massachusetts, for several years, begs to make the following report:

Several meetings of the committee were held at Woods Hole during the past summer, during which a thorough examination was made of the stored material. It was found to consist for the most part of letters and miscellaneous papers preserved by the various secretaries of the society during the period from 1899 to 1918. These were for the most part in the form of scrapbook volumes as follows:

- a. American Morphological Society, 1899-1900.
- b. American Society of Zoölogists, Eastern Branch, 1900-1902.
- c. American Society of Zoölogists, Eastern Branch, 1905-1908.
- d. American Society of Zoölogists, Eastern Branch, 1908-1910.
- e. Records and correspondence of Central Branch of American Society of Zoölogists previous to May 1, 1912.
- f. American Society of Zoölogists, 1913-1918.

In addition to these scrapbook volumes, there were various printed lists of members, programs of the annual meetings of the Society, and miscellaneous records of various treasurers.

After a thorough examination of the material and consultation with various members of the Society at Woods Hole, it was decided to go through the records and discard material that was obviously of no value. This was done, except in the cases where the letters were pasted in the scrapbooks, the removal of which would have injured the volume. In the material retained there is undoubtedly a considerable number of letters that can never be of value, but the amount of space occupied by such is inconsiderable.

The committee felt that the library of the Marine Biological Laboratory would make an ideal repository for this material, and the matter was taken up with the librarian, Mrs. Th. H. Montgomery. It was found that she was very ready to cooperate and at once made arrangements to store the material where it will be available at any time for the use of the members of the Society who are properly authorized. Furthermore, additional papers or volumes may be deposited as may be necessary in the future. The committee unanimously recommends: 1) that the library of the Marine Biological Laboratory be made the depository for the records of the Society and, 2) that all retiring officers of the Society turn over such correspondence and records, as seem advisable, to the secretary and that he prepare the material for depositing in the Marine Biological Laboratory Library.

Signed,
EDWIN LINTON,
CHARLES PACKARD,
GEORGE A. BAITSELL, *Chairman*.

Report of Nominating Committee

The committee (J. T. Patterson, Will Scott, T. C. Nelson) recommended the election of the following officers and representatives:

For president, one year, CHARLES ZELENY.

Vice-president, one year, J. H. BODINE.

Member of Executive Committee for five years, W. C. CURTIS.

Society representative on Type Culture Committee of National Research Council for three years, D. H. WENRICH.

Society representatives on the A.A.A.S. Council, one year, G. A. BAITSELL, W. H. LONGLEY.

Society representatives on Council of Union of American Biological Societies, one year, G. T. HARGITT, A. F. SHULL.

Members of the editorial board of Journal of Morphology for three years, L. R. CLEVELAND, F. L. HISAW, FRANZ SCHRADER.

There being no nominations from the floor, the Secretary was instructed to cast one ballot for the nominees. This was done and the nominees announced as elected. Vice-President Heilbrunn appointed the following members as a Nominating Committee for 1933: E. A. Adolph, S. Hughes-Shrader, and L. L. Woodruff.

ELECTION OF NEW MEMBERS

The Executive Committee recommended the election of 15 new active members, 8 associates to active membership, and 29 new associate members. The Secretary was instructed to cast one ballot for the names recommended. This was done and the nominees announced as elected, as follows (former associates elected to active membership are indicated by an asterisk (*)):

ACTIVE MEMBERS

Adamstone, F. B.	*Grant, Miss M. P.	Lewis, F. T.
*Andrews, J.	*Gregory, P. W.	*Pincus, G.
Barbour, T.	*Hurst, C. T.	Raffel, D.
Becker, E. B.	Hutt, F. B.	Schotté, O.
Bigelow, H. B.	*Jahn, T. L.	Weiss, P.
Breder, C. M., Jr.	Kozelka, A. W.	*Welsh, J. H.
*Cole, E. C.	Kropp, B.	Whiting, Mrs. A. R.
Cowdry, E. V.	Lambert, W. V.	

ASSOCIATE MEMBERS

Alexander, E. G.	Hetherington, W. A.	Nuttycombe, J. W.
Boughton, D. C.	Illick, J. T.	O'Roke, E. C.
Clausen, H. J.	Ingles, L. G.	Prosser, C. L.
Coatney, G. R.	Jeffers, Miss K. R.	Root, R. W.
Daniel, G. E.	Jones, E. R.	Rudnick, Miss G. S.
Dennis, E. W.	Lindeman, V. F.	Sawin, P. B.
Greeley, J. R.	Lynch, J. E.	Tressler, W. L.
Hall, E. K.	MacLennan, R. F.	Waterman, A. J.
Henry, Miss D. P.	McNeil, Miss E.	Yancy, P. H.
Herrick, E. H.	Noble, A. E.	

MISCELLANEOUS BUSINESS

The Executive Committee recommended the appointment of the following committees and the Society voted to instruct the incoming President to appoint them: *a.* Committee to consider future meeting places, with special reference to the agreement between the Zoölogists and the Botanical Society of America.

b. Committee to consider the future of the American Society of Zoölogists, with special reference to changes in organization and programs.

c. Committee to consider the printing of the annual program, proceedings, list of members, constitution, etc., by The Wistar Institute.

d. Committee to consider and formulate a financial policy for the Society.

The following resolutions were proposed and adopted by the Society:

a. The American Society of Zoölogists will cooperate with Section F of the A. A. A. S. in arranging the zoölogical program at the 1933 Chicago meeting in conjunction with the Century of Progress Exhibition, and in acting as host for the foreign zoölogists who are to be the guests of the A. A. A. S. Members of the American Society of Zoölogists will be appointed by the President to act as the Society's representatives at the meeting.

b. WHEREAS, the Joint Resolution of Congress creating the U. S. Bureau of Fisheries, approved February 9, 1871, provided for the appointment of a Commissioner of Fish and Fisheries who shall be a person of proved scientific and practical acquaintance with the fisheries; and

WHEREAS, For the past 20 years this practice has been followed, regardless of the party in power;

Therefore, be it resolved, That this Society urges the continuance of this policy as being the one means for assuring the upbuilding of our fisheries, and that a copy of this resolution be forwarded to President-elect Roosevelt.

c. The American Society of Zoölogists desires to emphasize the need for coordinated research in all phases of forestry and in the factors affecting the forest; not only trees, but associated herbaceous and shrubby vegetation, fungi, insects, and other animals, soils, and their organisms, and geological formations. Fundamental research in forest biology, which requires funds to insure protracted investigations, can appropriately be conducted in institutes of forest biology. Such institutions have been recommended by a committee of the Society of American Foresters and by I. W. Bailey and H. A. Spoehr in "The Role of Research in the Development of Forestry in North America." The Society believes that the establishment of fully equipped and adequately financed research institutes of forest biology will contribute materially to the advancement of science.

Upon recommendation of the Executive Committee it was voted to continue the payment of \$3 by each member to Biological Abstracts, but to reduce the annual dues for 1933 to \$1.

In response to a request from F. Payne, chairman of the Division of Biology and Agriculture of the National Research Council, the Society considered the two proposals for reorganizing the Division as presented by the Executive Committee of the Division. After discussion, it was voted to approve the second proposal, which embodies present practice, and to transmit the vote to the Executive Committee of the Division.

A vote of thanks to the local committee, the A. A. A. S., and the Ambassador Hotel for their cooperation and assistance in arranging for the Society's convenience and pleasure at Atlantic City was passed.

The Executive Committee announced that the 1933 meeting will be held in Boston with the A. A. A. S.

The meeting then adjourned.

WILLIAM H. COLE,
Secretary.

AMERICAN SOCIETY OF ZOÖLOGISTS

CONSTITUTION

ARTICLE I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoölogists."

Sec. 2. The object of the Society shall be the association of workers in the field of zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical science.

ARTICLE II

MEMBERSHIP

Section 1. The membership of the Society shall consist of two classes:

a. Associate membership open to all actively engaged in the field of zoölogy who have had training in zoölogy equivalent to that required for the doctorate degree.

b. Active membership open to those who hold the doctor's degree in zoölogy or who have done work equivalent thereto, and who, in addition to the usual requirements for this degree, have published the results of substantial zoölogical research.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Associate members shall not hold office or vote, but may present papers before the Society without introduction. Associate members shall receive all literature issued by the Society, including the proceedings of the Society, and shall have the same privileges as active members in journal subscriptions.

Sec. 4. Associate members may be elected to active membership in the usual way when the research requirements of active membership have been met.

ARTICLE III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary, a Treasurer, and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer, and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices. (See by-laws.)

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled, until the next annual meeting, by appointment by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee consisting of three active members. It shall be the duty of this committee to nominate officers of the Society, including the permanent representatives which the Society is asked to name to various boards and councils. This committee shall make its nominations to the Secretary at least four months before the next annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society, the time and place of the annual meeting shall be determined by the Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

ARTICLE V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of the Executive Committee.

ARTICLE VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society upon approval of two-thirds of the members voting, subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

c. The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

d. Members unable to attend may submit their votes in writing to the Secretary.

BY-LAWS

ARTICLE I

DUES

The annual dues for active and associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

ARTICLE II

PRESIDENT

Section 1. The President shall preside at scientific sessions and at the business meetings.

Sec. 2. He shall also appoint the committees prescribed by the constitution and by-laws.

ARTICLE III

VICE-PRESIDENT

Section 1. The Vice-President shall preside at sessions designated by the President.

Sec. 2. He shall also assume the duties of the President in the latter's absence.

ARTICLE IV

SECRETARY

Section 1. The Secretary shall keep the records of the Society.

Sec. 2. He shall be responsible for all arrangements for the meetings, including the appointment of local committees.

Sec. 3. Whenever the proper officers of a number of related societies shall have a conference covering matters of mutual interest to the several societies, he shall act as the delegate or representative of this Society.

Sec. 4. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society, and

Sec. 5. He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

ARTICLE V

TREASURER

Section 1. The Treasurer shall be in charge of the funds of the Society.

Sec. 2. At the annual business meeting of the Society he shall present a statement to date of the funds of the Society.

Sec. 3. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society.

ARTICLE VI

AUDITING COMMITTEE

The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

ARTICLE VII

PROGRAM RULES

In matters relating to programs for annual meetings, the following rules shall be observed:

Section 1. Titles and abstracts of papers to be presented by any method at the annual meeting must be sent to the Secretary in duplicate before the final date set by him. The length, form, and arrangement of the abstracts must comply with the requests of the Secretary in order to insure prompt publication by The Wistar Institute. The titles and abstracts of papers which do not conform to such requests or papers which are received late may be presented by title only.

Sec. 2. Titles shall be listed in groups according to subject matter, the groups to be determined by the Secretary.

Sec. 3. Whenever conditions require, the Secretary shall schedule two or more groups for the same hour and arrange the program to bring together papers on subjects of more general interest for meetings of the whole Society.

Sec. 4. Each member may have not more than fifteen minutes of the time of the Society at the annual meeting. This time may be used by him to present one or more papers personally or to introduce papers of non-members.

Sec. 5. All papers not read when called for as listed shall be placed at the end of the group list, and if not read when called for the second time, shall be read by title only.

Sec. 6. The titles of 'introduced' papers may be listed in the groups after the titles of papers to be read by members.

LIST OF FORMER OFFICERS

AMERICAN MORPHOLOGICAL SOCIETY

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1890—E. B. Wilson		J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

Additional Members of the Executive Committee

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

AMERICAN SOCIETY OF ZOÖLOGISTS

EASTERN BRANCH	<i>President</i>	CENTRAL BRANCH
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

LIST OF FORMER OFFICERS

EASTERN BRANCH	<i>Vice-President</i>	CENTRAL BRANCH
Jacob Reighard	1903	H. F. Nachtrieb
W. E. Castle	1904	S. J. Holmes
William Patten	1905	William A. Locy
William Patten	1906	George Lefevre
F. H. Herrick	1907	H. B. Ward
H. S. Jennings	1908	M. F. Guyer
H. V. Wilson	1909	M. F. Guyer
H. H. Wilder	1910	H. F. Nachtrieb
H. E. Crampton	1911	R. H. Walcott
G. A. Drew	1912	C. M. Child
Alex. Petrunkevitch	1913	C. M. Child

Secretary-Treasurer

G. A. Drew	1903	Frank Smith
G. A. Drew	1904	F. R. Lillie
H. S. Pratt	1905	C. E. McClung
H. S. Pratt	1906	T. G. Lee
C. J. Herrick	1907	T. G. Lee
L. L. Woodruff	1908	T. G. Lee
L. L. Woodruff	1909	Charles Zeleny
H. W. Rand	1910	H. V. Neal
Raymond Pearl	1911	H. V. Neal
J. H. Gerould	1912	W. C. Curtis
Caswell Grave	1913	W. C. Curtis

Executive Committeemen

F. R. Lillie	George Lefevre
T. H. Montgomery	T. G. Lee
H. C. Bumpus	Herbert Osborn
H. S. Jennings	C. H. Eigenmann
E. A. Andrews	J. G. Needham
W. R. Coe	S. J. Holmes
G. A. Drew	W. A. Locy
M. M. Metcalf	C. M. Child
D. H. Tennent	R. H. Walcott
R. G. Harrison	W. C. Curtis
H. E. Jordan	Oscar Riddle
C. E. McClung	H. B. Ward
	Chauncey Juday
	H. W. Norris
	C. E. McClung
	H. F. Nachtrieb

AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

	<i>President</i>	<i>Vice-President</i>	<i>Executive Committeemen</i>
1914.	C. E. McClung	M. F. Guyer	H. E. Jordan—1 year H. F. Nachtrieb—2 years H. V. Wilson—3 years George Lefevre—4 years A. F. Shull—5 years
1915.	W. A. Loey	W. E. Ritter	D. H. Tennent—5 years
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1917.	M. M. Metcalf	Charles Zeleny	M. M. Metcalf—5 years
1918.	George Lefevre	L. L. Woodruff	George Lefevre—5 years
1919.	C. M. Child	H. H. Wilder	C. M. Child—5 years
1920.	Gilman A. Drew	Caswell Grave	G. A. Drew—5 years
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1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

CENTRAL NATURALISTS

1899—Chicago	1900—Chicago
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SOCIETY OF AMERICAN ZOÖLOGISTS

1901—Chicago	1902—Washington
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1904—Philadelphia	1908—Baltimore	1905—(Mch.) Chicago
1906—New York	1911—Princeton	1907—(Mch.) Madison
1907—New Haven	1912—Cleveland	1907—Chicago
1909—Boston	1913—Philadelphia	1910—(Apr.) Iowa City
1910—Ithaca		1910—Minneapolis
		1912—(Apr.) Urbana
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1915—Columbus	1921—Toronto	1927—Nashville
1916—New York	1922—Boston	1928—New York
1917—Minneapolis	1923—Cincinnati	1929—Des Moines
1918—Baltimore	1924—Washington	1930—Cleveland
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TOTALS

Life members,	5
Active members,	502
Associate members,	126
Grand total,	633

THE CELLULAR CONTENT OF CHYLE IN RELATION TO LYMPHOID TISSUE AND FAT TRANSPORTATION

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ONE PLATE (SIX FIGURES)

Those lymphatics of the mesentery which are known as lacteals and which convey chyle away from the intestine after a meal rich in fat are similar to the lymphatics of the rest of the body in structure. They differ from the latter in appearance because of their marked change from a condition of transparency in the fasting animal to one of white opacity in the fat-fed animal. Furthermore, they are unique in so far as their relationship to lymphoid tissue is concerned. They stream in great numbers over the surfaces of the mesenteric lymph nodes which, for the most part, constitute a mass at the root of the mesentery in several of the commonly used laboratory animals. In this position, before passing through any lymph node, they may be spoken of as prenodal lacteals. Fewer efferent or postnodal lymphatics lead away from the lymph nodes and form one or more trunks passing to the thoracic duct. Lymphoid tissue, in the form of mesenteric lymph nodes, is thus interposed in the course of the lymph from the lacteals to the thoracic duct. The prenodal lacteals are significantly different from the primary afferent lymphatics in an extremity, which collect lymph directly from tissues normally containing few or no collections of lymphoid cells. The former, on the contrary, collect lymph from the intestine, which not only has a diffuse sort of lymphoid tissue in the mucosa, but also possesses aggregations of lymphoid

tissue in the form of nodules. Sometimes these occur alone as solitary nodules and sometimes in clusters as in a Peyer's patch.

Since the lymph nodes release cells into lymphatic vessels, the question arises as to whether the peripheral lymphoid tissue of the intestinal wall can do the same. If it can, one might expect to find numerous cells in the lymphatics afferent to the mesenteric lymph nodes and practically none in the primary afferent lymphatics of an extremity. The present study is an attempt to determine whether, and to what extent, this is true in the case of the mesenteric lymphatics. In addition, it seemed desirable to study the cells found in mesenteric lymphatics; and to compare them with the cells in the postnodal lymphatics.

With regard to the cellular content of lymphatics afferent to lymph nodes, very little information is obtainable from the literature. According to Maximow ('27), the lymph in the beginnings of the lymphatic system, in the lymph capillaries, contains practically no formed elements, but after passing through a lymph node it contains numerous lymphocytes. Haynes and Field ('31) studied the cell content of dog lymph, but did not determine in all cases whether or not the lymph had passed through a node. They found that lymph from the vessels in the femoral triangle contained from 0 to 2,500 cells, with an average of 520 cells. They attributed the presence of cells in these vessels to the fact that the lymph may have passed through a few small nodes at the back of the leg. Three samples of lymph obtained at the ankle showed no cells, 100 cells, and no cells, respectively.

Much work has been done on the cellular content of lymph other than that afferent to lymph nodes, but often the authors were not specific as to the exact source of the lymph with respect to lymphatic tissue. The relationship between the two is clear, however, in the following reports.

The leucocyte content of a normal efferent lymphatic draining an axillary lymph node was studied by Menkin and Freund ('29) in a dozen rabbits. The absolute counts varied

greatly, ranging from 9,000 to 55,000 leukocytes per cu.mm. of lymph. Supravital neutral red studied showed the cells to consist only of lymphocytes in 8 rabbits, 0.5 per cent monocytes in 2 cases, and in the remaining 2 the differential count was not given. The cellular content of lymph from the rabbit's thoracic duct just before it joins the venous system was studied by Kindwall ('27), who found these cells to average about 32,000 per cu.mm. and to be practically all lymphocytes. Davis and Carlson ('09-'10) have called attention to the fact that, following massage, the number of cells in thoracic duct lymph was immediately increased from 2 to 5 times. There was no change, however, in the relative number of the kinds of cells. After prolonged massage the cells of the lymph tended to decrease in number. Florey ('27) cannulated the lymphatic issuing from the mesenteric mass of lymph nodes in fat-fed cats, and studied the cellular content of the chyle obtained. His cell counts varied from 1,400 to 36,875 per cu.mm. Care was taken to avoid mechanical interference, but, even so, the counts varied greatly when successive samples from the same vessel were studied. Thus in one experiment a count made immediately after the insertion of the cannula gave 8,600 per cu.mm. Following a 15-minute interval, a second count gave 1,400 cells per cu.mm. In another experiment evidence was obtained that slight pressure, such as from a finger lightly applied, was capable of causing a great increase in the number of cells expelled from the nodes.

Cats were used in the present study, because the Peyer's patches are spaced rather far apart and solitary follicles are infrequent.¹

Although the Peyer's patches lack the sinuses found in lymph nodes, injection experiments showed that their nodules were surrounded by lymphatic vessels. However, a study of sections cast some doubt upon the possibility of cells passing into these surrounding lymphatics as easily as they could into the peripheral and central sinuses of a lymph node.

¹ A review of the knowledge of the anatomy of lymphoid tissue in vertebrates, including the cat, is given by Oppel (1897).

The cellular content of the lymph in the vessels concerned in the present study was investigated by two general methods, one involving the counting of the cells in a measured quantity of fresh lymph, and the other in the study of fixed and stained preparations.

In the cat, the lacteals afferent to the mesenteric lymph nodes were found to be very small. When they contained little chyle, as in the fasting animal, it was usually impossible to obtain enough fluid by any method to count cells. Capillary pipettes were used to get chyle from the larger efferent lacteals, but these were found unsatisfactory for the afferent channels, both because their ends tended to wound the vessel wall and to pick up contaminating cells, and because the chyle in such small amounts tended to cool and become semisolid before it could be expressed on to a cover glass and drawn up into a calibrated pipette for counting. When the vessels were distended, following a meal rich in fat, even the smaller lacteals of the living cat exuded chyle onto the free surface of the mesentery when cut across with a sharp knife. The freely exuded chyle was drawn into blood cell counting pipettes, diluted and thoroughly mixed.

It was necessary in securing exuding chyle to bear in mind the possibility of contamination in the following ways: 1) By blood from the abdominal incision, or from capillaries ruptured when the lacteal was cut across; 2) by free cells in the peritoneal cavity, and, 3) by loosened mesothelial cells. In addition, there was the probability that, with the passing of time, the necessary exposure and manipulation of the intestines would cause an inflammatory reaction. Hence the time element was thought important, and a schedule of the time of obtaining the chyle was made. The use of killed animals would have obviated this difficulty, but a free flow of chyle could not have been obtained.

The cats used for study of fresh samples of chyle were starved 36 to 72 hours and then given all the whipping cream and mutton fat they would take. The studies were made 10 to 12 hours later. Ether was given and the animals were

kept alive while the samples were being collected. Care was exercised in haemostasis when the abdominal cavity was opened, the cut edges of the abdominal wound were protected with warm saline packs, and the intestine was handled very gently. Saline was never used to wash off the intestine, lest the fluid influence the dilution of cells. As soon as a loop of intestine began to dry, it was replaced in the abdominal cavity. Usually the lacteals contained milk white chyle from near the pylorus to within about 10 cm. of the ileocaecal valve. The terminal portion of the ileum usually showed translucent lacteals, but the occasional occurrence of gray lacteals, and the microscopic demonstration of fat particles in lymph from this region indicated the difference in fat absorption between this and more proximal levels of the intestine to be one of degree only. After a lacteal was cut across with a sharp knife, enough fluid could be obtained to fill the ordinary white blood cell counting pipette to between the 0.1 and the 1.0 mark. The chyle was then diluted with 1 per cent acetic acid and the cells counted at a magnification of 400. It was found possible to divide them into three groups: 1) Polymorphonuclear leucocytes, 2) mesothelial cells with rounded nuclei, and, 3) others, also with rounded nuclei, which were not mesothelial cells. A second drop of chyle from the same source was used to make a dried film, which was later stained with Wright's stain. As soon as the specimen was obtained, a tag was sewed in the mesentery to mark the source. The taking of each specimen was timed from the moment the animal became unconscious from the effect of the anaesthetic.

After the counts were made the intestine was opened and the Peyer's patches located. Any Peyer's patch in the neighborhood of one of the marked sites was injected with 2 per cent Berlin blue in aqueous solution, to see if it drained into the site. After fixation, the intestine was cut into free-hand sections, stained, and examined for solitary or aggregated nodules. Blocks were taken for microscopic sections.

The results of counting cells of fresh chyle are given in table 1. The table has been arranged to facilitate a compari-

TABLE 1

CELLULAR CONTENT OF CHYLE AS DETERMINED BY COUNTING FRESH SAMPLES

CHYLE FROM PRENODAL LACTEALS							CHYLE FROM POSTNODAL LACTEAL	
DRAINING NO PEYER'S PATCH				DRAINING A PEYER'S PATCH				
CAT NO. AND WT. IN KG.	DISTANCE ABOVE ILEO- CAECAL VALVE, CM.	TIME IN MINUTES	WHITE CELLS PER CU. MM.	DISTANCE ABOVE ILEO- CAECAL VALVE, CM.	TIME IN MINUTES	WHITE CELLS PER CU. MM.	TIME IN MINUTES	WHITE CELLS PER CU. MM.
I	68		933					15175
2.1 kg.	55		2500					
II	65	5	1430	14	20	5673	35	19910
2	32	10	292	25	25	5170	40	5673
kg.	69	15	110					
	29	30	165					
III	53	10	602	1.5	14	47200	45	18771
1.4	18	20	277				50	14003
kg.	29	24	771					
	38	28	1157					
	68	35	500					
	25	40	1023					
IV	8	10	175	41.5	19	3200	50	1690
2.7	34	15	264	46	24	550		
kg.	62	28	120	4.5	39	1462		
	28	34	340	12	43	300		
				78	48	3510		
V	50	13	2000	7	10	2200	37	7315
2.0	97	20	330	76	17	1600		
kg.	110	23	286					
	116	27	300					
	93	33	400					
VI	13	5	333				dead	12222
1.0	35	10	666				"	5360
kg.	51	13	248					
	57	17	1025					
	79	22	2084					
VII	3	23	1666	13	12	9454	17	152224
1.0	9	27	1110	40	31	1639	43	9640
kg.	51	34	333					
	54	40	0					
VIII	66	17	1200				30	143800
2.2							34	129800
kg.								
Average			730				6829	41199
*Correction for free								
peritoneal cells			31				31	
Corrected average			699				6798	41199

*See Text

son between the number of cells in chyle from prenodal lacteals and chyle from postnodal lacteals. The former have been divided into two groups according to their relationship with a Peyer's patch. The origin of the postnodal chyle with respect to Peyer's patches was impossible to determine, because lacteals from numerous sources passed to the same lymph node. The table also includes information on the distance from the ileocaecal valve to the level where the chyle was obtained. In connection with these data it should be stated that the length of the small intestine in these cats was usually 100 to 125 cm.

The ratio of red cells to white cells in the stained smears, determined for each sample of chyle taken, is not included in the table. It was obvious from a study of this ratio that there was not merely a contamination of normally cell free chyle with blood from ruptured capillaries or from the abdominal incision. Only in one instance was the ratio in smears of chyle more than half as much as the normal ratio (about 500:1 in cats) of red cells to white cells in the circulating blood. The total absence of red cells from smears in a number of samples of chyle, in some cases taken after the abdomen had been open for a long time, denied their presence simply as the result of manipulation of the intestine. The fact that there was no constant increase in the number of red or white cells during the process of taking samples also indicated that inflammation had no great effect upon the presence or the ratio of the cells. Furthermore, differential counts, to be mentioned later, also supported the belief that the chyle, as obtained, was contaminated neither by blood per se nor by cells due to manipulation, since it was found to contain largely cells with round nuclei, and very few polymorphonuclear cells. This condition was the opposite of that found in the blood stream, and it could not be taken as indicative of an acute inflammatory reaction.

To control the results given in table 1, with respect to contamination from cells of the peritoneal moisture normally present on the mesentery and from any cells which might

appear there during the process of the collection of samples, two cats were treated in exactly the same manner as the preceding, except that the lacteals were not cut open. A drop of indifferent fluid, either physiological saline or blood serum obtained by heart puncture from the same animal 2 days previously, was placed on the surface of the mesentery to simulate the drop of exuding chyle. The cells which the drop of indifferent fluid had attracted from the mesenteric surfaces were then counted in the usual manner. When the average number of cells found was subtracted from the average number of cells recorded in table 1, it was found that contamination with free peritoneal cells was an insignificant factor.

It was believed from a consideration both of the precautions taken to prevent contamination of the samples of chyle and of the data secured to check on the possibility of contamination, that the figures given in the table represent with a fair degree of accuracy the actual cellular concentration in the lacteals of the intact animal.

The great variation in the counts had been expected from a study of the literature. Counts which appeared atypical in that they were much higher than other counts in the same group were due to no definite pathological lesion which could be demonstrated. Thus in cat no. 3, there was a count of 47,200 cells per cu.mm. in a lacteal springing from a Peyer's patch. Microscopic sections through the Peyer's patch showed no definite lesion. In cat no. 8 the high counts on post-nodal chyle suggested the possibility of an infectious lesion or a leukaemia. Microscopic sections of the lymph nodes from which the lacteal drained, and of the liver, spleen, and other organs failed to show such a lesion. The atypically high counts are to be explained on a basis either of mechanical pressure on lymphoid tissue when the fresh samples were secured or of a chronic inflammatory process of low degree not readily demonstrable. This second possibility will be discussed later.

Regardless of individual variations, the counts done on chyle from each of the three sources showed unmistakable trends. The chyle which had passed through lymph nodes contained the greatest number of cells per unit volume of fluid. That which had passed through and around a Peyer's patch contained an intermediate number of cells, and that which had come from the intestinal wall away from a Peyer's patch contained the least number of cells, sometimes almost none. As possible sources for the last mentioned cells are the very rare solitary follicles in the cat and the diffuse lymphoid tissue of the mucosa.

An analysis of the average figures obtained revealed that chyle which had passed through and around collections of compact lymphoid tissue in the intestinal wall, i.e., the Peyer's patches, contained approximately ten times as many cells per unit of volume as chyle which had not. It was concluded that the cells in the chyle which had been in such close contact with lymphoid tissue on part of its pathway had actually left the lymphoid tissue and floated free in the chyle. This was borne out by examination of sections through Peyer's patches. Dilated spaces lined with endothelium, and partly filled with white cells, occasionally could be found in the submucosa just external to the Peyer's patches (fig. 5). The vessel was considered a lymphatic if it contained no red cells and if most of the cells had round nuclei. Search for similar vessels of smaller caliber in the mucosa internal to Peyer's patches revealed cell-free vessels, but here interpretation of their exact nature was more difficult. Similar dilated spaces in the submucosa not near any Peyer's patch never contained many cells. Hence it seemed more than likely that large numbers of cells were derived from Peyer's patches and shed into the passing stream of chyle.

A second group of fat fed cats, six in number, was used to prepare fixed sections of lacteals for study of the comparative number and types of cells in chyle from the various sources by another method, one which excluded the possibility of contamination of chyle or of changes in its cellular content

due to handling the mesentery. These cats were killed and their abdominal cavities filled with Zenker-formol fixative. Following fixation, blocks of the mesentery containing lacteals were taken at various levels of the small intestine, and of the intestine itself at the point at which the lacteal in question appeared to emerge. At least three blocks were taken in the course of each lacteal, except in a few cases in which its length permitted only one. Approximately half a dozen serial sections were taken from three different levels of each block. The sections were cut at $10\ \mu$ and stained with azure II-eosin and haematoxylin as used by Maximow.²

The distribution of cells along the course of a given lacteal was found to be fairly uniform. Isolated red cells were found within the sectioned lacteals, but with exceeding rarity. The number of white cells in each lacteal appearing in each section was counted and the diameter of the lacteal in question was approximated with the aid of stage micrometer and camera lucida. By the use of a suitable equation, the data at hand yielded a rough approximation of the number of cells per cubic millimeter of chyle. To determine the source of chyle with respect to the Peyer's patches, the block taken from the apparent source of a lacteal was studied, and in addition gross stained serial sections of the intestine above and below that point were studied with the binocular dissecting microscope for the presence of lymphoid nodules.

Since the number of cells per cubic millimeter of chyle determined in this way was only an approximation of the true number present, the exact figures obtained are not given. In general, however, they showed the same trends as the counts of fresh chyle, namely, that chyle from prenodal lacteals which did not drain a Peyer's patch contained very few cells, and that chyle from prenodal lacteals coming from a Peyer's patch contained many more cells, the number of cells in the chyle from the latter source being, in fact, almost as great as the number of cells in postnodal chyle.

² This method is detailed by Slider and Downey ('29).

Figures 1 to 6 show photomicrographs of sections of lymphatics containing chyle from the various sources. The cellular content of these lacteals is to be noted.

A study of the data secured in the course of these experiments revealed that in the case of lacteals draining Peyer's patches, those of a gray color contained twenty-four times and the translucent ones seventeen times as many cells as did the white lacteals. In the case of lacteals not draining Peyer's patches, the results were of the same nature, but the differences between lacteals of varying color were much smaller. The impression was gained, therefore, that the cellular content of chyle was inversely proportional to the fat content.

This finding lends no support to the commonly expressed view that leucocytes, and especially lymphocytes, are probably important agents in fat transportation. It is clear that they cannot be important in carrying out such a function in chyle, for every sample of chyle, especially when not coming from a Peyer's patch, showed an insignificant number of cells in relation to the large quantity of fat. Whether leucocytes carry fat particles from the intestinal epithelium through the tissue of the villus and into the central lacteal, as Schäfer ('20) has described, was a problem not included in the present work.

In the case of vessels coming from the intestine, especially from collections of lymphoid tissue, the possibility of chronic and subacute infections of the intestinal wall, even in apparently healthy animals, must always be considered. For this reason a gross study of the intestinal contents was made in nearly every case, and in most cases microscopic sections of intestinal wall, mesenteric nodes, and other organs were examined. In all cases gross sections were made through these organs to see whether tubercles and abscesses were present. No positive evidences of active inflammatory processes were found in the intestinal walls, or mesenteric nodes. The presence of secondary nodules within the lymphoid nodules of the Peyer's patches was not used as a sign of inflammation. The

secondary nodules originally described as germinal centers may be, following the ideas of Hellman ('30), centers of reaction to irritants which normally begin to enter the tissues of all animals shortly after birth. The possibility of some such 'normal' process of chronic inflammation is fully recognized. It may well be that the great variation in cell counts in the present study is due in part to this factor.

Supravital studies, using neutral red and Janus green, were made of the chyle of three additional cats and differential counts were made of the dry smears and sectioned lacteals from all cats used.

The majority of the cells of chyle, whatever its source, were lymphocytes. Prenodal chyle not from a Peyer's patch showed the greatest proportion of cells other than lymphocytes. The composition of prenodal chyle from a Peyer's patch was very similar to that of postnodal chyle, indicating that peripheral lymphoid tissue was, in some respects, functionally analogous to the tissue of lymph nodes not only because both released cells into lymphatics, but also because the cells released were of the same type.

SUMMARY

A study of chyle in the domestic cat showed that it almost always contained white cells before passing through any lymph node. Although the number of cells per unit volume of chyle varied greatly, chyle which had passed through and around Peyer's patches contained on the average many more cells than chyle which had not. Furthermore, the number of cells in chyle was inversely proportional to the large amount of fat present. This indicated that they had little to do with fat transportation in the lacteals. Chyle after passing through a lymph node contained, as a rule, more cells per unit volume than prenodal chyle. Wherever found, the cells of chyle were predominantly lymphocytes. The peripheral lymphoid tissue of the cat's small intestine appears to be analogous to a lymph node in respect to its capacity to release lymphocytes into lymphatic vessels.

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PLATE 1

EXPLANATION OF FIGURES

The lacteals figured were selected to show the average cellular content found in serial sections. The distribution of cells within prenodal lymphatics was fairly uniform, but was more varied in the larger postnodal channels.

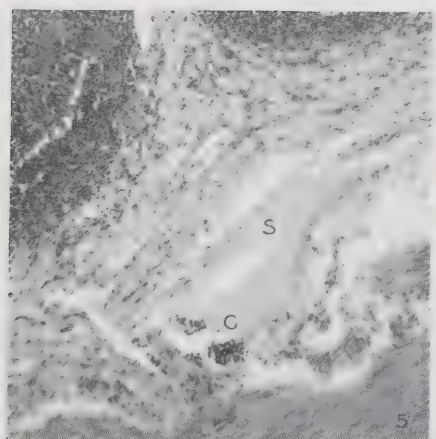
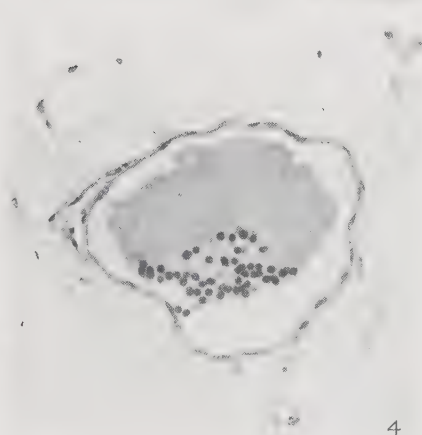
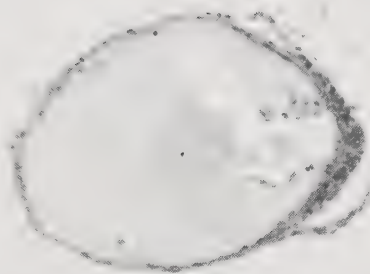
1 Two prenodal lacteals presenting the appearance which they often had in sections, that is, devoid of cells. These lacteals did not drain a Peyer's patch.

2 The appearance of an occasional prenodal lacteal, i.e., containing a few cells. Two of the three cells present are in focus, and all are small lymphocytes. This lacteal does not drain a Peyer's patch.

3 and 4 Lacteals which drain Peyer's patches and contain many small lymphocytes.

5 A submucosal lymphatic, *s*, containing many cells, *c*, which are mostly small lymphocytes. A second, smaller, lymphatic showing a few cells lies just to the left of the larger one. Parts of two nodules of a Peyer's patch lie above them.

6 A postnodal lacteal. Most of the cells, which are gathered at one end, are small lymphocytes.



ABNORMALITIES IN THE BRAIN STEM ASSOCIATED WITH MALFORMATION OF THE EAR

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THREE FIGURES

At times those fortuitous malformations which occur during embryonic development yield information difficult to obtain by the experimental approach. Too often these are slighted at the routine necropsy. The present report, concerning a deficiency in the brain stem correlated with an absence of the homolateral external ear and malformation of the internal and middle ear, illustrates the necessity for following up such opportunities.

Several reports upon similar cases have appeared in the literature. They differ in one or more important details; however, such is to be expected, since the combination of causes and their time of onset must vary to culminate in different patterns. Thus, the aforementioned approach is rendered more valuable since, given equally accurate appreciation of the individual pattern, it should be possible in the future to fit all together into a complete research.

CASE HISTORY AND NECROPSY RECORD

A premature (8 lunar months) male infant was born after a normal labor in the Chicago Lying-in Hospital. At birth, absence of the left external ear and canal was noted. The child died 40 hours later. At necropsy, performed after 3 hours, terminal pneumonia was assigned as the cause of death. Dr. E. W. Hagens, of the Department of Otolaryngology of Rush Medical College, requested a special necropsy of the defective ear and brain.

The family history was negative as regards the present condition. The father was healthy and denied having had venereal disease. The mother was healthy, about 24 years of age and had had no serious or significant past illnesses. She had had one previous pregnancy, $1\frac{1}{2}$ years before the present one, which terminated in the stillbirth of a premature (9 lunar months) infant. The mother's Wassermann reaction was negative at each pregnancy.

Doctor Hagens' report of the findings in the malformed left ear is, in part, as follows:

The outer ear, external canal and drum membrane were absent. The malleus, incus and stapes were malformed. The tensor tympani muscle was present; the stapedius absent. Two abnormal veins were present in the middle ear and followed the course taken by the third and fourth portions of the seventh nerve when present. A mass of thymic tissue was situated in the lower part of the middle ear. The end organs of the cochlea and vestibular apparatus were normal. The spiral ganglion and cochlear root were absent. Scarpa's ganglion and fibers were definitely present, but not normal in number. The seventh nerve, geniculate ganglion and chorda tympani nerve were absent. Several ganglion cells were found off the apex of the cochlea¹ and a definite collection at the front end of the petrous bone. Nerve fibers were present in two tracts which were located in the area of the petrosal nerves. The internal auditory meatus was narrowed and its aperture filled with connective tissue so that there appeared to be no association of nerve fibers with the brain. No asymmetries were noted in the brain.

METHOD

The brain was received after it had been injected with 10 per cent formalin and immersed in that fluid for a month. A detailed examination revealed no asymmetries or other abnormalities. One rootlet was identified in the area of the VII-VIII complex upon the left side. The right VIIth and VIIIth nerves were of normal caliber. The left IXth, Xth,

¹ The possibility that this collection represented a malformed geniculate ganglion must be kept in mind.

XIth, and XIIth nerves were identified and proved to be of equal size with those of the normal right side.

The brain stem from a level slightly above the medial geniculate body to one below the pyramidal decussation was mordanted in Müller's fluid for a period of one month, washed, dehydrated and embedded in paraffin. Serial sections, 15 μ in thickness, were mounted 6 or 8 to the slide. Every fourth slide throughout the series was stained with azure I and erythrosin, the remainder by Morgan's ('26) iron hematoxylin method. Preliminary study showed that fixation had been extremely poor above the level of the Vth nerve nuclei. Below this level it was adequate for purposes of study.

OBSERVATIONS

1. Cochlear nerves, primary and secondary nuclei and connections

Upon the normal right side the cochlear division of the VIIIth nerve occupied its normal position relative to the dorsal and ventral cochlear nuclei and the restiform body. At its entrance to the brain its greatest transverse diameter was 0.6 mm. The majority of fibers spread out from the lateral surface of the nerve to be distributed in the ventral nucleus. Other fibers were seen to continue into the dorsal cochlear nucleus. On the left side, study of serial sections did not reveal a cochlear nerve or even isolated fibers of one in its usual site. Figure 1 is a photograph, made at a low magnification, of the area under discussion.

The dorsal and ventral cochlear nuclei were normally represented upon the right side. Each received its full complement of fibers. The area which should have been occupied by these nuclei upon the left side was greatly reduced so that the restiform body encroached upon the dorso-lateral aspect of the brain stem. A small remaining area, corresponding to the lateral portion of the right ventral cochlear nucleus, was made up entirely of glial cells. Despite the absence of the left ventral cochlear nucleus, occasional fibers

could be traced into this area from the reticular formation of the medulla.

The size, position, and relations of the superior olivary nuclei were not different upon the two sides. Each had a normal cell and fiber distribution (fig. 3). The nuclei of the trapezoid body were also apparently equally developed. Cell groups corresponding to the nucleus praeolivaris and nucleus semilunaris (Barker, '01) could not be distinguished upon either side.

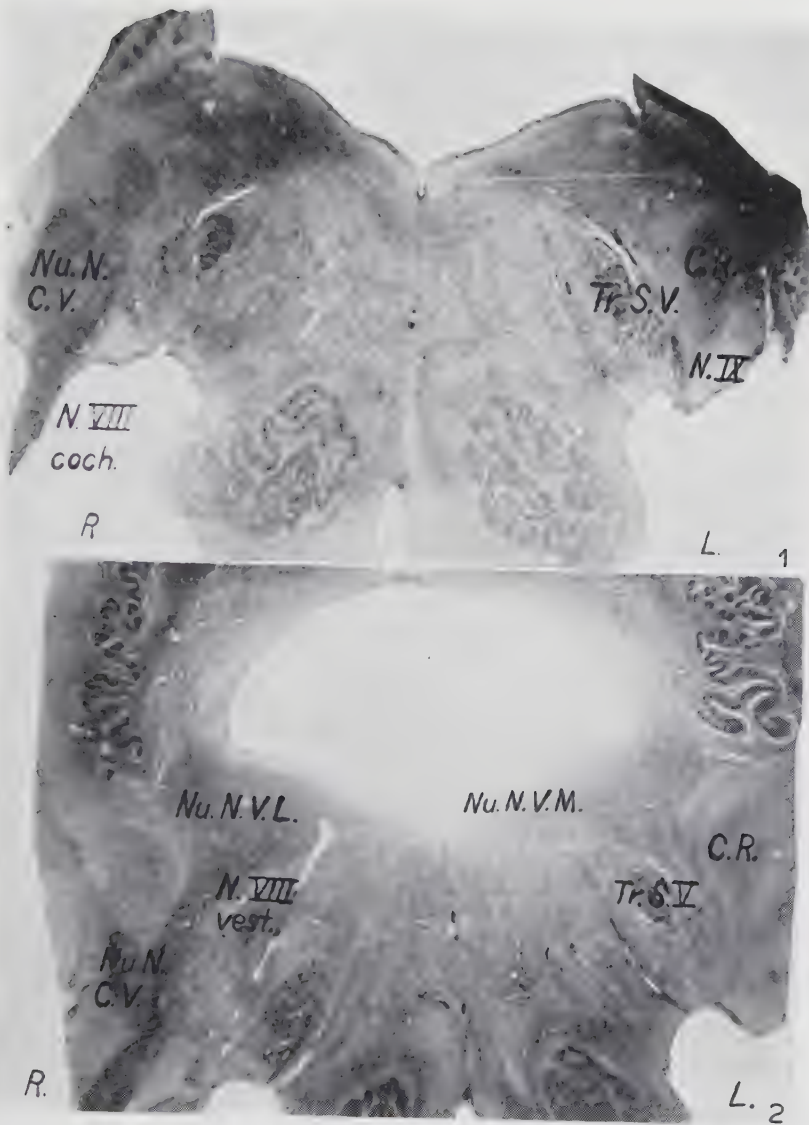
The fibers of the trapezoid body were equally numerous upon the two sides of the median raphe. Monakow's decussation was also bilaterally represented. Unfortunately, the preparation did not show the lemnisci very clearly.

2. Vestibular nerves, nuclei, and connections

The vestibular division of N. VIII, like the cochlear division, was not represented upon the left side (fig. 2). No fiber bundle, or even isolated fibers, was to be found in a position comparable to that occupied by the normal right nerve. However, upon the left side, at a level corresponding to the caudal limit of the entry zone of the right N. cochlearis, a bundle of fibers was observed which coursed from the postero-lateral sulcus to the region of the tractus solitarius. Following this path it passed between the lateral border of the spinal tract of the trigeminal nerve and the ventro-medial aspect of the restiform body. Though somewhat cephalic to the usual position of entry of the N. glossopharyngeus, there seems no doubt that this nerve was in fact the glossopharyngeal.

Fig. 1 Photograph of cross section of the brain stem at the level of entrance of the cochlear division of the VIIIth nerve. *Nu.N.C.V.*, ventral cochlear nucleus; *N.VIII, coch.*, cochlear nerve; *Tr.S.V.*, spinal trigeminal tract; *C.R.*, restiform body; *N.IX*, glossopharyngeal nerve. *L*, left or abnormal side; *R*, right or normal side. Note absence of cochlear nerve fibers or primary nuclei upon the left side. Morgan's iron hematoxylin method.

Fig. 2 Cross section somewhat anterior to that of figure 1, showing the entrance of the vestibular branch of the VIIIth nerve. *Nu.N.V.L.*, lateral vestibular nucleus; *Nu.N.V.M.*, medial vestibular nucleus; *N.VIII, vest.*, vestibular nerve. Note the absence of the vestibular division upon the left side. Morgan's iron hematoxylin method.



Figures 1 and 2

The superior, median, and lateral vestibular nuclei were present on the right and left sides. No apparent differences in cell content were noted when like nuclei from the two sides were compared. The spinal nucleus was not identified upon either side. The vestibulo-cerebellar connections were equally well developed.

3. The fifth, sixth, and seventh cranial nerves

The Vth and VIth cranial nerves were normally and equally developed bilaterally. The VIIth right nerve was normal and all of its component divisions could be identified. The left motor facial nucleus was hypoplastic; approximately $\frac{1}{10}$ to $\frac{1}{20}$ as many cells could be identified as were present in the normal right nucleus. Its pars prima and pars tertia were represented by isolated fibers, the pars secunda could not be identified. The left sensory VIIth was present, however, and apparently normally developed. Its fibers could be traced through the lateral portion of the spinal trigeminal tract. They were lost shortly thereafter (fig. 3).

DISCUSSION

Of chief interest in the analysis of these observations are: 1) the unilateral involvement, thus enabling comparison of the normal and maldeveloped sides; 2) the presence of normal cochlear and vestibular end organs coincident with aplasia of the cochlear nerve and ganglion, hypoplasia of Scarpa's ganglion and its fibers peripherally, aplasia of the vestibular nerve centrally; 3) the apparent discrepancy between the normal development of the primary vestibular nuclei and absence of the primary auditory nuclei; 4) the occurrence of hypoplasia in the motor facial nucleus and nerve upon the maldeveloped side with a well-developed sensory seventh. Parallel cases in the available literature do not afford such a combination of circumstances.

The reported cases of congenital deafness in which the brain findings have been established naturally fall into the categories of positive and negative observations. We are

here concerned with those which show aplasia, hypoplasia, or atrophy in the primary auditory and vestibular locus. Of these, two excellent reports by Brouwer serve as examples with which the remainder may be compared: The first ('12) concerned the brain of a congenitally deaf cat, the second ('14, with von Walree) that of a woman deaf and dumb from birth. In each, the involvement of the brain was bilateral;

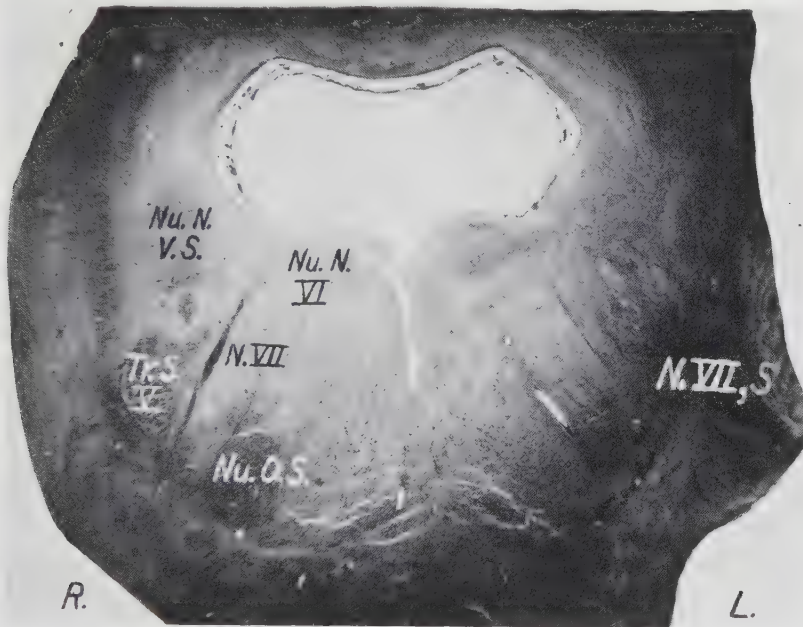


Fig. 3 Cross section at level of the facial colliculus. *Nu.N.VI*, nucleus of abducens nerve; *Nu.N.V.S.*, superior vestibular nucleus; *N.VII,S.*, sensory root of the VIIth nerve; *Nu.O.S.*, nucleus of the superior olive. Morgan's iron hematoxylin method.

there was complete absence of the cochlear roots, high grade atrophy (or aplasia) in the primary auditory nuclei, diminution in the lemniscus fibers (Monakow's decussation, the trapezoid body, and the lateral lemniscus). The vestibular nerves and primary vestibular nuclei were not malformed in either case. Significant changes were likewise lacking in the 'superior olivary complex.'

The cases reported by Castex and Marchand ('06), Siebenmann and Bing ('07), and Zanca ('08), like those of Brouwer, showed atrophy in the primary cochlear nuclei. Castex and Marchand, and Zanca found diminution of fibers in the trapezoid body; Siebenmann and Bing found it to be normal. Zanca also reported a diminished number of fibers in the lateral lemnisci.

The combination of circumstances found in the brain herein described gives opportunity for several interpretations, no one of which, in the light of our present knowledge, is in complete accord with all of the observations. The primary issue involved is the differentiation of aplasia from atrophy. The absence of any residues upon the malformed side, which might have represented normally developing cochlear nuclei of an earlier phase of intra-uterine life, is favorable to the interpretation of aplasia. Further evidence is the absence of the external ear and canal, which would indicate a defective otic plate, the coincident hypoplasia of the motor facial nucleus, and the absence of an external factor (tumor or abnormal vascular growth) which might explain atrophy. Favorable to the interpretation of atrophy is the finding of well-developed cochlear and vestibular end organs and a group of nerve cells which undoubtedly represented a vestige of Scarpa's ganglion. This would indicate that the acustico-facial ganglion had developed, since the origin of an entire group of such cells from the otic placode would seem highly doubtful (Bartelmez, '22); but such a possibility is not eliminated. The weight of evidence, therefore, favors aplasia as the primary condition, although in the sequence of development it would seem likely that atrophy became associated with it.

The coincidence of normally developed vestibular nuclei and absent vestibular nerve, with absent primary auditory nuclei and nerve is a discrepancy previously alluded to. In Brouwer's cases the vestibular nuclei were also normal, but there they were associated with a normal vestibular division. The answer to this question may lie in the diversity of sources

from which stimuli influence the developing vestibular as compared with the developing primary auditory nuclei.

Hypoplasia of the motor seventh nerve upon the malformed side probably has the same explanation as underlies the previously discussed deviations from pattern. The presence of a normal sensory seventh in such circumstances would not be unusual, since Bartelmez (*loc. cit.*) considers it certain that at least a part of the geniculate ganglion in man is derived from the epibranchial placode of the hyoid arch.

The apparent lack of difference in the fiber content of Monakow's decussation and the trapezoid body upon the two sides is not significant when the unilaterality of the involvement is remembered. In those cases reported in the literature showing a significant diminution in the numbers of fibers of these groups, there was bilateral involvement. Neither is significance attributed to the normal superior olive of the malformed side.

SUMMARY

The brain findings are reported in a premature infant of 8 lunar months, born without the left external ear and canal, with maldeveloped middle and internal ears. The right ear was normal. The auditory and vestibular roots, the dorsal and ventral cochlear nuclei were absent upon the side of the maldeveloped ear. Upon the same side the motor facial nucleus and nerve were hypoplastic, the sensory seventh apparently normal.

I wish to acknowledge my indebtedness to Dr. E. W. Hagens, who generously afforded me the opportunity of reporting the brain findings in this interesting case. At times, in the interests of brevity, it has been essential to consider Doctor Hagens' observations with my own. All observations and interpretations concerning the structure of the ear were made by him. I am also indebted to Dr. G. W. Bartelmez, of The University of Chicago, and to Dr. Lorente de No, of the Central Institute for the Deaf, St. Louis, for helpful criticism.

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CYCLIC HISTOLOGICAL VARIATIONS IN THE ANTERIOR HYPOPHYSIS OF THE ALBINO RAT¹

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ONE FIGURE

The work of Rasmussen ('21) first suggested that there are cyclic histological changes in the anterior hypophysis which may be correlated with the different phases of the oestral cycle. This investigator found that, in the spring, as the woodchuck comes out of the dormant state and enters the rutting season, there is an increase in the number and size of the basophilic cells in the anterior hypophysis.

Somewhat later, Charipper and Haterius ('30) reported cyclic histological changes in the anterior hypophysis of the rat, which they correlated with the phases of the oestral cycle. They stated that during late dioestrus both basophiles and eosinophiles were present. In late oestrus basophiles and a 'few' poorly stained eosinophiles were found. In late dioestrus the basophiles had a compact nucleus and a meager amount of light blue cytoplasm, while in late oestrus the blue cytoplasm of these cells was more abundant.

Recently, Reese ('32) has reported that during oestrus the eosinophiles of the hypophysis of the rat are well filled with granules which stain intensely, while in dioestrus the granules of these cells are less closely packed and stain less intensely.

In previous papers (Wolfe, Cleveland, and Campbell, '32 a; Wolfe, Cleveland, and Campbell, '32 b; and Cleveland and

¹ This work has been supported by a grant from the National Research Council Committee for Research in Problems of Sex.

Wolfe, '32 b) we presented evidence which indicated that there are cyclic histological changes in the anterior hypophysis of the dog and the domestic sow, and that these histological variations can be correlated with the various phases of the oestral cycle. It was also found that there are characteristic differences in the number and appearance of the cell types which we feel are referable to the species. It was tentatively suggested that these differences in the cell types of the anterior hypophysis can be in a general way correlated with the type of oestral cycle exhibited by the particular animal (Cleveland and Wolfe, '32 b).

In the anterior hypophysis of the dog, three granular and one non-granular cell types were differentiated. One of the granular types was acidophilic and was classified as type I. It was considered identical to the eosinophile or alpha cell commonly described in the literature. The other two granular types were basophilic in nature and were considered similar to the commonly accepted basophile of the literature. These two types were designated as types II and III, respectively. The non-granular type was considered identical to the chromophobe or chief cell. It was tentatively concluded that the granular cells, types I, II, and III, lost their granular material and passed into the cells of type IV (non-granular chromophobes).

In the sow only two granular types were differentiated. The cells of the first type were acidophilic and were considered identical to the cells of type I, found in the dog. The second type of granular cell was basophilic and was in every way similar to the basophiles of the literature. This cell embodied characteristics of both of the basophilic types (types II and III) found in the hypophysis of the dog, and, although similar to, was not identical to either of these cells; however, it was designated as type III. The non-granular cell type found in the anterior hypophysis of the sow was identical with type IV of the dog.

In the anterior hypophysis of the dog, during the pro-oestral phase, the relative percentage of the cells of type III

reached a high level (10.2). A majority of these cells contained granular material which stained purple-red. In animals killed in oestrus, and which had ovulated, it was found that the percentage of these cells was increased to 11.7. However, practically none of these cells contained granular material. During the subsequent lutein phase these cells were few in number, contained no granular material, did not appear active, and at the end of this period made up only 2.2 per cent of the cells present. In oestrus, the cells of type I were packed with granules which stained a deep yellow. By the end of the lutein phase of the cycle, the relative percentage of these cells was markedly decreased (from 50.2 to 42.5). The granules in those which did remain were loosely packed and scattered. They stained a pale yellow. In the oestral glands the relative percentage of the cells of type II (second basophilic type) was 7. The closely packed granules stained a deep red. By the end of the lutein phase of the cycle, the relative percentage of these cells was reduced to 2. The few cells of this type remaining exhibited marked reduction of granules. During the lutein phase of the cycle, parallel to the drop in the percentage of the granular cells, there was an increase in cells of type IV to the extent that at the end of the lutein phase they outnumbered the granular cells, from 30.9 per cent in oestrus to 53 per cent at the end of the lutein phase. During the subsequent anoestral period there was a marked increase in the percentage of the granular types, with a parallel decrease in the relative percentage of the non-granular type.

Similar cyclic histological changes were observed in the anterior hypophysis of the sow. The cells of type III which were completely filled with granular material reached a level of 9 per cent in prooestrus. In the oestral phase of the cycle the relative percentage of these cells which were completely filled with granular material was extremely low (4.8 per cent), while the number which did not contain granular material was correspondingly high. During the early lutein phase, before organization of the corpora lutea was complete, the cells

of type III which were completely filled with granular material reached a high level of 9.2 per cent. By the end of the lutein phase, the percentage of these cells which contained granular material was reduced to 2.6. Throughout this phase, these cells appeared regressive; i.e., the cytoplasm was fragmentary and the cell membrane was indistinct. It was thought that they passed into cells of type IV.

During oestrus, the cells of type I reached a relative percentage of 50.5. They contained granules, closely packed in the cells, which stained intensely with orange G. By the end of the subsequent lutein phase, the granular content of these cells was much reduced. The granules remaining stained lightly with orange G. There was also a reduction in the relative percentage of these cells throughout the lutein phase, from 50.5 per cent to 42.1 per cent. There was an increase in the cells of type IV from 20.6 per cent in oestrus to 34.5 per cent at the end of the lutein phase. Since it was possible to discern cyclic histological changes in the anterior hypophyses of dogs and sows which could be correlated with the different phases of the oestral cycle, it seemed desirable to extend these studies to the albino rat. The rat has been used extensively in research on the anterior hypophysis, and it was considered of importance to determine the cyclic histological changes in the anterior hypophysis of this animal in order to form a basis for subsequent experimental studies.

MATERIAL AND METHODS

Due to the excellent studies of Long and Evans ('22), the cyclic histological changes in the ovaries and the accessory reproductive organs of the rat are well known. By the application of the vaginal smear technic of Stockard and Papanicolaou ('17) to the rat, these investigators found that the rat exhibits a short cycle, 4 or 5 days in length. Ovulation is spontaneous; the subsequent corpora lutea remain inactive unless copulation occurs.

By use of the vaginal smear technic we have obtained anterior lobe material from forty rats, killed at selected periods

during the oestral cycle. In each instance, however, the exact period in which the animal was killed was verified by histological study of serial sections of the ovaries and representative sections of the uteri and vaginae.

The method of preparation of the anterior lobe material has already been described (Cleveland and Wolfe, '32 a) and will not be repeated here.² Each gland studied has been cut serially in the horizontal plane at 1.5 to 2 μ . One section out of each ribbon of twenty-five was mounted. In this way sections from all levels of the gland have been obtained for study. We feel that this is of greatest importance because of the tendency of the different cell types to aggregate in different areas of the gland. Using the method introduced by Rasmussen and Herrick ('22), we have made comparative cell counts on every section studied, and every fifth field in the section was counted. Although it cannot be claimed that this method gives an exact ratio of the different cells present in the section, it seems probable that it gives the most reliable data obtainable on the relative percentages of the various cell types present in the section.

Cell types found in the anterior hypophysis of the rat

In general, it may be said that there are two granular and one non-granular cell types found in the anterior hypophysis of the rat. A third granular (basophilic) type has occasionally been observed.

The first granular type to be described, type I, is in every way identical with the cells of type I found in the anterior hypophysis of the dog and the sow, and to the eosinophiles of the literature. Large and small cells were included in this group, although intermediate forms were present. In the smaller cells the nucleus was prominent. It was usually light and vesicular and was identical in size to the nuclei found

² Anterior lobe material from rats was more difficult to stain than that from dogs and sows. It was found advantageous to use 5 per cent potassium dichromate, rather than 3 per cent, as a mordant for the sections after they were mounted on the slide. The mordant was changed daily for 3 days. The staining time in orange G and aniline blue was 8 and 10 minutes, respectively.

in the larger cells of this type. The nucleus was surrounded by a narrow band of cytoplasm, which as a rule appeared only slightly granular, and stained deeply with orange G. The cell membrane was usually distinct.

In the larger cells the large and distinct granules were usually less closely packed and stained less intensely with orange G, although these were by no means invariable findings and varied in respect to the phase of the oestral cycle in which the animal was killed. In a majority of the larger cells the cell membrane was fairly distinct. In some instances cells of this type were observed which contained only a small amount of granular material. In such cells we observed a normal appearing nucleus surrounded by a small number of pale yellow granules. The cell membrane of such cells was usually quite indistinct. The cytoplasm of the cells of type I was usually colorless, although in some instances a few strands of pale blue cytoplasm were observed. Since we observed a certain number of these cells in which the granular material was sparse and was scattered about the nucleus in an irregular fashion, and since the cell membrane was often indistinct, the impression was gained from the study of these cells that they lost their granular material and passed into the non-granular cell type (type IV). It must be emphasized, however, that we never observed great numbers of these cells in any one section, which had lost a great amount of their granular material. We have, however, in a single section, observed a great number of these cells in which the granular material was somewhat reduced.

Two types of basophilic cells were observed, although one of these was found only infrequently. The basophilic cells found invariably in every section were quite similar to the cells of type III found in the anterior hypophysis of the dog. Therefore, we have classified this basophilic type in the rat as type III. This cell type was found in two forms: cells which were completely filled with basophilic granules and cells which either had a small amount of granular material or no granular material at all.

The granular forms included small and larger cells. The nuclei in both were similar, light and vesicular. The granules which were suspended in a light blue cytoplasm were extremely fine and usually stained a dark blue, although in a few instances they stained violet. The granules were usually so closely packed in the cell that they gave the impression of a dense blue cytoplasm. A clear area or macula, such as that described by Rasmussen, was usually evident in the cells completely filled with granular material. In the smaller cells the nucleus was surrounded by a narrow band of granular material, while in the larger cells the granular mass was more abundant.

These cells apparently lost their granular material and gave rise to a specific non-granular form³ of the cells of type III; i.e., large cells with light blue cytoplasm and an intact cell membrane. Often in these large blue cells a small amount of the granular material of the cells of type III was observed. This granular material was seldom observed in a clumped condition, as was the case in similar cells in the anterior hypophysis of the dog. In the rat it seemed that the granules of the cells of type III became less closely packed and eventually disappeared from the cell altogether, giving rise to the large, clear blue cells. These cells persisted temporarily; then there was a fragmentation and, later, an almost total disappearance of the light blue cytoplasm. The cell membrane became invisible and it was thought that these cells passed into the cells of type IV, the common non-granular form.

It is tentatively suggested, therefore, that the two granular cell types lose their granular material and pass into non-granular forms. It appears that the cells of type I pass

³ Our observations would indicate that these cells are a specific non-granular form of the cells of type III and appear as the result of loss of granular material from the cells of type III. They are large cells with blue cytoplasm and an intact cell membrane. Therefore, we have listed them as cells of type III which contain no granular material. They should not be confused with the cells of type IV (chromophobes), a cell type which we have considered as a common non-granular form. However, they apparently undergo regressive changes and give rise to the cells of type IV. These regressive changes consist of fragmentation of the cytoplasm and disappearance of the cell membrane.

directly into the cells of type IV, while the cells of type III lose their granular material and give rise to a specific non-granular form; i.e., large cells with light blue cytoplasm and an intact cell membrane. However, this specific non-granular form undergoes regressive changes and passes into cells of type IV.

A second type of basophilic cell which was occasionally observed had nuclei similar to those of the cells of type III. The granules of these cells were large and uniform, and stained a deep red. They were usually closely packed in the cell. No cells of this type were observed in which there was a diminished amount of granular material. These cells resembled the cells of type II of the dog.

The non-granular cell type, classified as type IV in this paper, found in the anterior hypophysis of the rat was in every way identical to the chromophobes of the literature and to the cells of type IV of the dog and the sow. They were usually observed as nuclei surrounded by a few strands of pale blue cytoplasm. In many instances no cytoplasm at all was demonstrable. The cell membrane was usually invisible. These cells were found either clumped together in masses or scattered among the granular forms. The cells were rarely observed bordering on the capillaries.

We were unable, by the use of our staining technique, to differentiate the cells of type IV; that is, we could not tell whether any certain cell of type IV arose from a cell of type I or from a cell of type III. However, Severinghaus ('32), by the use of the Golgi technique, reports that such a differentiation may be made. In this paper, because of the limitation of our staining technique, we have considered the cells of type IV (chromophobes) as a common non-granular type.

*Cyclic histological changes in the anterior hypophysis
of the rat*

In the anterior hypophysis of the dog and of the sow, cyclic histological changes were described in the hypophysis which were both quantitative and qualitative. By quantitative

changes we mean the changes in the relative percentage of the various cell types present at the different phases of the cycle. By qualitative variation, we point to the intrinsic cytoplasmic changes that took place in the cells themselves. In the dog both the quantitative and qualitative changes were marked, while in the sow the qualitative changes predominated.

In the rat, a species which exhibits an extremely short cycle and one in which the corpora lutea do not ordinarily become active, it was found that the cyclic histological changes were for the most part qualitative; there was only slight evidence of a quantitative cycle. In this paper we have considered material taken from animals killed in prooestrus, oestrus, metoestrus, and dioestrus.

The anterior hypophyses of rats killed in prooestrus

Material has been collected from six rats killed in the prooestral phase of the cycle (stage 1 of Long and Evans). In these glands it was found that the cells of type I, although somewhat variable in the actual relative percentage of cells, averaged about 32 per cent. There were usually large numbers of the small cells of this type; i.e., cells with a narrow band of granules surrounding the nucleus. The granular material of these cells usually stained intensely with orange G. The cell membrane was distinct. There were also larger cells of this type, all of which stained intensely with orange G. As a rule, cells of this type, in which the granular material was scattered and which stained lightly with orange G, were not observed.

The cells of type III usually made up 4 to 6 per cent of the cells present. A majority of them were larger cells, completely filled with fine granules, which stained heavily with aniline blue. These cells were the largest observed in the rat glands. A lesser number of cells of type III, which contained no granular material (that is, large blue cells with intact cell membrane and blue cytoplasm) was observed.

The cells of type IV predominated in every phase of the cycle and were quite abundant in prooestrus. In this phase they were usually observed as cells which were clumped together in large groups. Table 1 shows the relative percentage of the various cell types found in this phase of the cycle.

The anterior hypophyses of rats killed in oestrus

Material was obtained from twelve animals killed in oestrus. The percentage of the cells of type I was usually high in oestral glands. The granules of both the small and large cells were usually closely packed in the cell and stained heavily with orange G—a finding already reported by Reese ('32).

The cells of type III were essentially different from those found in prooestrus. In early oestrus, there were about an equal number of cells of type III which contained granular material and those which contained no granular material. In late oestrus (stage III of Long and Evans), one seldom observed cells of type III which contained granular material. One did, however, observe large numbers of the non-granular form of this cell; i.e., large clear cells with a light blue cytoplasm, which at this time often exhibited regressive changes (fragmentation of the cytoplasm). The cell membrane was usually intact.

Although the cells of type IV predominated, they were usually less abundant in this phase of the cycle than in any other. Table 2 shows the relative percentage of the various cell types found in this phase of the cycle.

The anterior hypophyses of rats killed in metoestrus

In our experience, rats in this laboratory have usually exhibited a metoestrus smear for 1 or 2 days after oestrus. Since the animals were smeared only once daily, it is impossible to determine the exact length of this period. However, all animals used invariably exhibited a dioestrus smear on the third day after oestrus, and many on the second day.

In the animals killed on the first day after oestrus, the granules of the cells of type I, in contrast to the condition found in oestrus, were usually less closely packed in the cell

TABLE 1
Hypophyses of rats killed in prooestrus

RELATIVE PERCENTAGE OF CELL TYPES PRESENT						SECTIONS STUDIED	CELLS COUNTED
ANIMAL NUMBER	Type I	Type III			Type IV		
		Granular	Non- granular	Total			
NC-257	27.0	5.2	0.6	5.8	67.2	4	1,885
NC-26-C	32.4	4.4	0.2	4.6	63.0	6	2,602
NC-251	27.2	4.1	0.5	4.6	68.2	5	3,522
NC-13-C	39.9	0.8	4.2	5.0	55.1	5	2,439
NC-2	41.1	4.2	1.7	5.9	53.0	6	3,759
NC-331	29.0	4.0	0.1	4.1	66.9	4	2,316
Average cell % for phase	32.8	3.8	1.2	5.0	62.2	33	Total 16,523

TABLE 2
Hypophyses of rats killed in oestrus

RELATIVE PERCENTAGE OF CELL TYPES PRESENT							
ANIMAL NUMBER	Type I	Type III			Type IV	SECTIONS STUDIED	CELLS COUNTED
		Granular	Non- granular	Total			
NC-70	24.8	2.2	3.4	5.6	59.6	4	2,638
NC-25-C	31.4	0.6	5.9	6.5	62.1	4	2,245
NC-18-C	37.2	0.7	7.2	7.9	55.5	5	2,677
NC-77	31.5	1.0	5.5	6.5	62.0	6	4,291
NC-14	33.4	2.1	5.4	7.5	59.1	5	2,392
NC-79	30.6	5.8	1.1	6.9	62.5	5	4,504
NC-256	24.5	1.2	4.5	5.7	69.8	4	3,067
NC-7	37.2	0.5	7.3	7.8	55.0	6	6,026
NC-320	34.5	1.0	3.5	4.5	61.0	3	1,009
NC-232	29.1	0.0	5.3	5.3	65.6	4	2,320
NC-313	35.0	0.2	3.4	3.6	61.4	4	2,683
Average cell % for phase	31.7	1.4	4.7	6.1	61.2	50	Total 33,852

and perhaps stained lighter with orange G. This was not a constant finding, however. On the second day after oestrus (second day of metoestrus or first day of dioestrus) this finding was more constant, although not invariable. This is essen-

tially the same condition as that found by Reese ('32). A few cells of this type were observed in which the granular material was markedly diminished. In such cells the reduced amount of granular material was arranged about the nucleus in an irregular fashion.

The changes in the cells of type III were more marked. Practically no cells of this type were observed which contained granular material. The non-granular form of this cell, large oval cells with light blue cytoplasm, appeared regressive. In a majority of these cells the light blue cytoplasm was broken up and arranged irregularly about the nucleus. The cell membrane was often indistinct. Such changes were more evident on the second day after oestrus than on the first day.

The cells of type IV (chromophobes) were usually abundant; the relative percentage was 62.4. Table 3 shows the relative percentage of the various cell types found in this phase of the cycle.

The anterior hypophyses of animals killed in dioestrus

Animals in this laboratory usually show a dioestral smear the second or third day after oestrus. This period usually lasts for 1 or 2 days. The cells of type I in animals killed in this period were variable. In the larger cells the granules were loosely packed and stained lightly with orange G. In the smaller cells the granules were closely packed and stained intensely with orange G.

The cells of type III were also variable. About 50 per cent of those present were of the smaller type and contained dark blue granular material. The others appeared regressive and contained no granular material. The blue cytoplasm of the latter cells was extremely fragmentary. The cell membrane was usually indistinct. Histological evidence suggested that these cells passed into the cells of type IV. The percentage of the cells of type IV was usually high. Table 4 shows the relative percentage of the various cell types found in this phase of the cycle. Thus it seems that in dioestrus we find

TABLE 3
Hypophyses of rats killed in metoestrus

RELATIVE PERCENTAGE OF CELL TYPES PRESENT							
ANIMAL NUMBER	Type I	Type III			Type IV	SECTIONS STUDIED	CELLS COUNTED
		Granular	Non- granular	Total			
NC-12-C	30.4	0.3	3.7	4.0	65.6	4	2,627
NC-68	34.8	0.4	3.5	3.9	61.3	5	4,578
NC-52	34.6	0.6	4.4	5.0	60.4	4	2,739
NC-78	37.0	1.9	4.6	6.5	56.5	4	2,373
NC-250	31.8	2.1	2.5	4.6	63.6	4	3,880
NC-80	33.4	0.0	6.5	6.5	60.1	5	3,112
NC-322	35.9	0.7	3.2	3.9	60.2	3	1,142
NC-319	35.9	0.2	3.3	3.5	60.6	4	3,380
NC-335	33.2	0.0	2.9	2.9	63.9	4	2,681
NC-338	32.1	0.2	2.7	2.9	65.0	4	2,832
NC-317	30.0	0.0	2.2	2.2	67.8	4	3,776
Average cell % for phase	30.3	0.5	3.7	4.2	62.2	45	Total 33,120

TABLE 4
Hypophyses of rats killed in dioestrus

RELATIVE PERCENTAGE OF CELL TYPES PRESENT								
ANIMAL NUMBER	Type I	Type III			Type IV	SECTIONS STUDIED	CELLS COUNTED	
		Granular	Non- granular	Total				
NC-243	38.3	6.2	0.4	6.6	55.1	4	1,158	
NC-53	33.7	1.8	0.5	2.8	63.5	5	3,967	
NC-4-C	36.0	4.6	0.2	4.8	59.2	4	2,668	
NC-9-C	27.3	2.0	2.7	4.7	68.0	5	2,928	
NC-254	28.2	1.1	2.5	3.6	68.2	4	3,166	
NC-76-A	27.1	2.0	2.1	4.1	68.8	6	4,451	
NC-69	30.0	0.4	1.8	2.2	67.8	4	1,692	
NC-326	33.5	2.2	2.2	4.4	62.1	3	909	
NC-327	36.0	2.4	3.3	5.7	58.3	4	2,202	
NC-325	28.0	3.8	2.2	5.0	67.0	5	2,269	
NC-324	40.6	1.6	1.3	2.9	65.5	4	2,821	
NC-17	21.9	2.3	3.5	5.8	72.3	5	5,468	
Average cell % for phase		31.8	2.5	1.9	4.4	63.8	53	Total 33,699

cells of type I which exhibit regressive changes and at the same time young cells of type I. The same was true when we consider the cells of type III. It seems possible that the older cells, which exhibit regressive changes, represent cells which were active in the previous cycle of the animal, and that the younger cells will be active in the next or subsequent oestral cycles.

DISCUSSION

It is of interest to compare the cyclic histological changes in the anterior hypophysis of the rat with those which occur in the anterior hypophyses of the dog and of the sow.

In all three species it was found that the cells of type I were identical and that they were also identical to the eosinophiles or alpha cells described in the literature. In the dog and the sow these cells undergo similar changes in the cycle. During oestrus they are well packed with granules which stain intensely with orange G. By the end of the subsequent lutein phase in the dog the relative percentage of these cells is markedly reduced (from 50.2 per cent in oestrus to 42.5 per cent at the end of the lutein phase). Those cells which remain exhibit a reduced number of granules which stain lightly with orange G. Coincident with the decrease in the percentage of the cells of type I there is an increase in the cells of type IV. In the lutein phase of the cycle of the sow similar changes were observed in the cells of type I. Thus it is seen that the lutein phase of the cycle of these two species is characterized by a decrease in the amount of granular material and by an actual reduction in the percentage of the cells of type I. However, in the rat we have an animal which does not exhibit a lutein phase in the normal cycle. In the rat there is no definite evidence of a quantitative reduction of the cells of type I in any phase of the cycle. There are, however, throughout the normal cycle of the rat, qualitative changes in the cells of type I which are similar in a limited degree to the qualitative changes which occur in these cells in the normal cycle of the dog and the sow. In hypophyses of rats killed in

oestrus these cells are well filled with granules which stain intensely with orange G. The cell membrane is always well defined. Two days later, the granules of these cells are somewhat more loosely packed in the cell and stain more lightly with orange G. The cell membrane may or may not be distinct.

Since in the dog and the sow, the lutein phase of the cycle is characterized by a reduction in the relative percentage of the cells of type I, it seemed possible that the induction of a lutein phase into the cycle of the rat would induce quantitative changes in the cells of type I comparable to those which occur in the lutein phases of the cycles of the dog and the sow. Examination of anterior lobe tissue from pregnant and pseudo-

AVERAGE PERCENTAGE OF CELL TYPES PRESENT						GLANDS STUDIED	SECTIONS COUNTED	CELLS COUNTED
PERIOD	Type I	Type III			Type IV			
		Granular	Non-granular	Total				
Prooestrus	32.8	3.8	1.2	5.0	62.2	6	30	16,525
Oestrus	31.7	1.4	4.7	6.1	61.2	11	50	36,054
Metooestrus	33.8	0.5	3.7	4.2	62.4	11	45	33,120
Dioestrus	31.8	2.5	1.9	4.4	63.8	12	53	33,699
							Total	
						40	178	119,396

Fig. 1 Summary of data on hypophyses from normal female rats.

pregnant rats during first 7 or 8 days of these periods reveals the fact that there is a marked reduction in the relative number of the cells of type I. In oestral glands the relative percentage of these cells is 31.7. They are well defined and are completely filled with granular material which stains intensely with orange G. At the end of the seventh day of pregnancy, the relative percentage of these cells is 27. The cells of this type which remain in the gland exhibit an extreme reduction of granular material, which takes a pale stain with orange G. Our experience with these cells in the normal cycle of the dog and sow and in pregnancy and pseudopregnancy in the rat would indicate that during an active lutein phase these cells are markedly reduced in granular content and in relative percentage in the gland.

When we consider the basophilic cell types in these three species, certain variations were found. Certain facts indicate that the variation of the basophilic cell types might be correlated with the type of oestral cycle exhibited by the animal.

In the dog, two basophilic types were observed: cells of types II and III. Furthermore, the dog exhibits an oestral cycle which extends over a long period of time. The oestral and lutein phases of the cycle are well separated; i.e., a period of several months separates the lutein phase of the cycle from the subsequent oestral phase. In this animal evidence was presented to show that the basophilic cell, type III, was associated, as far as time relations are concerned, with the oestral phase of the cycle, and the basophilic cell, type II, underwent loss of granular material in the lutein phase of the cycle.

In the anterior hypophysis of the sow, only one basophilic cell type was differentiated. However, it presented characteristics of both the basophilic types found in the anterior hypophysis of the dog. This cell was apparently active in both the oestral and lutein phases of the cycle of the sow. In other words, we had in the sow a single basophilic cell type, which apparently played the role which two basophilic cell types played in the oestral cycle of the dog. In this connection it must be remembered that the oestral cycle exhibited by the sow is essentially different from that exhibited by the dog. In the sow, the oestral and lutein phases of the cycle are closely connected; i.e., the lutein phase of the cycle is rapidly followed by the next oestral period. Although there is no conclusive evidence, it seems that this variation of the basophilic types of the two species might be explained by the difference in the oestral cycles.

In the rat we have usually observed only one basophilic cell type. This cell was essentially similar to the cells of type III of the dog, the basophilic type which was associated with the oestral phase of the cycle of the dog. It was in no way similar to the cells of type II of the dog, the basophilic

type of the dog which was associated with the lutein phase of the cycle of that animal. Thus, in the rat, which exhibits only an oestral phase in the normal cycle, a basophilic cell type is observed which is similar to the basophilic type of the dog, associated with the oestral phase of the cycle of that animal. The second basophilic type found in the anterior hypophyses of the rat was observed too infrequently to be considered here.

CONCLUSIONS

1. Evidence has been presented to show that there are cyclic histological changes in the anterior hypophysis of the rat. These variations may be correlated with the different phases of the oestral cycle.

2. These cyclic histological changes are qualitative rather than quantitative.

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OBSERVATIONS ON THE STRUCTURE OF THE BLOOD VESSELS WITHIN THE THYROID GLAND OF THE DOG

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TWO PLATES (SEVENTEEN FIGURES)

INTRODUCTION

During investigations on the innervation of the thyroid gland, Prof. José F. Nonidez ('31 a and '31 b) noted peculiarities in the vessels in the gland, some of which were suggestive of direct communications between arteries and veins. It was at his suggestion that the present study was undertaken, and the author expresses gratitude to Doctor Nonidez for assistance and criticism during the course of the work.

Aside from clinical papers on the collateral circulation of the thyroid and its significance in surgical conditions, the literature on the anatomy of the vessels of the thyroid is rather meager. A search revealed no statement concerning arterio-venous anastomoses in the gland. The existence of arterial anastomoses within the depths of the gland has been questioned by many and has been the subject of some controversy. Landström ('07) believed that arterial anastomoses were present beneath the capsule, but was unable to demonstrate them. Major ('09) studied the vessels of the thyroid gland in dogs by means of injection, clearing, and microdissection. He found numerous anastomoses on the surface, but was unable to identify any within the gland. However, because of the limitations of his methods for the demonstration of very minute vessels and because of the extreme vascu-

larity of the organ, he did not deny the possibility of their existence. Shigyo ('23) found arterial anastomoses uniformly present throughout the gland. Marine ('28) was of the opinion that there were no arterial anastomoses below the surface.

Several investigators have described the presence of endothelial buds or 'Knospen,' as termed by the Germans, in the smaller arteries of the thyroid gland. Horne (1892) found these in a number of fetal, newborn, and adult thyroids. He considered them to be formed by localized proliferations of endothelial cells. The structures were entirely separate from the muscle coat of the vessel. In some sections the buds appeared to lie free in the lumen, but a study of serial sections showed them to be pedunculated. In some thyroids the buds were much more numerous than in others, but in all they were absent in the larger arterial trunks. They were found most frequently at the point of branching of the smaller arteries. Schmidt (1894) found that these projections into the lumina of the arteries, consisting of endothelial cells, took on a variety of shapes. Some were almost spherical, some hemi-spherical, while others, which must have been pedunculated, appeared to lie free in the lumina of the vessels. From Schmidt's figures it is not possible to determine whether these structures are really of endothelial origin. The nuclei of the cells appear quite round in all sections and are surrounded by an ample layer of cytoplasm. The structures described by Schmidt were, as in the case of the other investigators, found only in the smaller arteries. The function ascribed to them is that of slowing the flow of blood so that it does not rush too quickly through the wide capillary bed. Schmidt found 'Knospen' normally in dog, cat, and human thyroids. Hesselberg ('10) described the 'Knospen' and also considered their origin as being endothelial. She found that these structures were always separated from the muscularis by the elastica interna. Her figures show this clearly and there can be little doubt that the structures figured are not composed of muscle cells.

The problem of the collateral circulation of the gland is not relevant to the present study and the reader is referred to a study by Kurkowsky ('30) on that subject and to one by Rabl ('22), who discussed in great detail the development of the blood vessels in the thyroid gland of the guinea pig. Both of these papers give extensive bibliographies.

METHODS

The thyroid glands from twenty dogs were studied. Nine were fixed in formalin (15 per cent), and frozen sections ($45\ \mu$) were cut and stained according to the Cajal ('25) silver nitrate, pyridine, and alcohol technique. The advantages of this method are that the endothelial cells and smooth-muscle cells of the arteries, as well as the nerve fibers, are well impregnated, whereas the follicular epithelium and connective tissue of the gland are only faintly visible. In these preparations, if the plane of section parallels the course of an artery, it is often possible, because of the thickness of the section, to follow the vessel for some distance. Capillaries, venules, and lymphatics are also well stained, because of the specificity of the treatment for endothelium. In one instance a fine precipitate of silver carbonate was injected through the superior thyroid artery before fixation. Frozen sections ($45\ \mu$) of the injected gland were cut, some were mounted without staining, while others were stained according to the Cajal technique. A solution of silver nitrate to which ammonium hydroxide was added until the formed precipitate just dissolved was introduced by gravity through the superior thyroid artery into two glands immersed in formalin (15 per cent). Frozen sections ($45\ \mu$) were cut, some were mounted without further treatment, and others were stained according to the Cajal technique. In the former case the cement between the endothelial cells of the vessels is impregnated, while no other structures take the stain. In such preparations the continuity of vessels is easily demonstrated.

In addition to this material, thyroids of dogs, impregnated with the methods of Golgi and the reduced silver nitrate of

Cajal (one series), were also studied. These were prepared by Doctor Nonidez for his investigation of the innervation of the thyroid. In the Golgi sections the endothelium is heavily impregnated with the silver, and the lumina of the vessels stand out as solid black lines. The muscle cells in the wall of the arteries can be identified. In the Cajal slides all structures may be easily identified. Finally, observations were also made on two series of thyroid sections stained with hematoxylin and eosin and one series stained by the Mallory method for elastic tissue (from the collection of Prof. Charles R. Stockard).

Most of the glands were from normal adult dogs, except the thyroids impregnated with the Cajal reduced silver nitrate method, which were from a puppy 7 weeks old. In two instances, in which the Cajal silver nitrate, pyridine, and alcohol technique was followed, the animals had previously been used in experiments on the action of iodo-acetic acid. These animals died with congestive heart failure, so that the smaller arteries and capillaries were engorged with blood and were extremely easy to define and follow. No injections were made with a view to corrosion preparations, clearing, or to the taking of Roentgenograms, because such methods do not allow the positive identification of the structure of the smaller vessels.

OBSERVATIONS

The distribution of the larger vessels in the thyroid is so well known that no consideration was given it in the course of this investigation. The reader is referred to the paper by Major ('09) for a discussion of the subject.

In the methods used in the present study, with the exception of the few serials, the finding of vessels for study depended largely on chance, on the angle at which the vessels were cut, and the inclusion of entire vessels within the thick sections used. However, the extreme vascularity of the gland (5.6 times as vascular as the kidney: Tschuewsky, '03) increased the chances for such fortuitous findings.

In cross sections of the smaller arteries, occasionally a definite bundle of cells, internal to the circular muscle layer, was found (figs. 1 and 1 a). These groups of cells push into the lumina of the vessels and are covered by the endothelial layer. Further study indicated that these structures bear a constant relationship to the branches of the smaller trunks within the gland, and they were found with great regularity in such positions. When the trunk and a branch of an artery are included in the plane of section in such a way that their continuity can be directly observed (figs. 2, 3, 3 a, and 4), these bundles of cells appear on both sides of the arterial branch at the point of junction with the trunk, and extend for a short distance along the walls of the trunk beneath the endothelium. They push into the lumen from both sides and appear, in section, as cushions, as they will be termed. In this position they seem, at times, to be large enough to occlude the lumen of the vessel.

When the cushion is cut just below the point of branching (figs. 1 and 1 a), the cells forming the cushions are seen in cross section, whereas the cells of the muscularis of the arteries appear circularly oriented. In longitudinal sections of the trunk the cells of the cushion appear as longitudinal fibers, whereas the muscle cells of the vessel wall are cut transversely. The cushions appear to be continuous with the circular muscle layer of the branch artery, and it would seem that the cells of the cushions are extensions of the layer of circularly oriented muscle cells from the branch into the wall of the trunk. These cells run longitudinally into the parent trunk as they leave the branch. This relationship is shown in figures 2 and 3 and can be more readily visualized if the smooth-muscle layers of an arterial trunk and its branch coming off at right angles are considered. Since the smooth-muscle layers of the arteries are circular, it will be seen that in such an instance the plane of a single smooth-muscle fiber in the branch lies at right angles to the plane of a single fiber in the trunk. Thus, if the smooth-muscle layer of the branch is continued into the trunk, these cells will appear at right

angles to the circular muscle layers of the trunk; i.e., they will appear as longitudinal fibers. Hence, in cross sections of an artery just below a branch, and which includes a cushion, both circular and longitudinal muscle fibers appear, the latter representing an extension of the muscularis of the branch into the trunk. It would appear as if the muscularis of the branch were telescoped into the trunk and formed cushions to allow for the continuity of the lumen.

A somewhat different arrangement of muscle fibers is seen in figure 4, which shows a cushion at the junction of an artery and its branch. The cells forming the cushion are continuous with the muscle layer of the trunk rather than of the branch. This latter type of cushion, however, is not frequently found.

Although in section the muscle bundles appear as cushions, they entirely surround the lumen of the branch as it leaves the arterial trunk. However, the muscle cells do not form a ring around the lumen, but continue for a short distance up and down the trunk along its longitudinal axis and appear to form a lip-like valve rather than one of the true sphincter type.

In the identification of the cushions as muscular structures two points must be established; namely, that they are not artifacts produced by a section cutting the trunk and the branch at a peculiar angle, and that the cushions are, in fact, composed of smooth-muscle cells. The former possibility may be ruled out, since not only were the sections so thick that entire vessels with their branches containing cushions were observed, but also some of these structures were studied in serial sections. That the cushions are anatomical entities cannot be denied.

Whether they are composed of endothelial cells, and are the 'Knospen' described by Horne, Schmidt, and Hesselberg, or of muscle cells, is a point of certain physiological significance, because the presence of muscle indicates activity on the part of an anatomical unit. The cells in the cushions are directly continuous with the layer of smooth-muscle cells of the arteries (figs. 2, 3, and 4). By all the methods used, the

cytoplasm of these cells stain similarly to the cytoplasm of the circular smooth-muscle cells forming the media of the arteries. The nuclei are essentially identical with the nuclei of the cells of the muscularis, which are larger and have finer scattered chromatin granules, and are quite distinct from the endothelial nuclei. The latter are more prominent and have the chromatin more concentrated in the periphery of the nuclei (fig. 4 a). Where the cushions are cut in cross section, the nuclei can be seen centrally placed within the cells. The nuclei in the cushions are elongated, whereas in Schmidt's figures the nuclei of the 'Knospen' are round regardless of the plane of the section. Moreover, in sections stained by the method of Mallory, the cells of the cushions were observed to be separated from the endothelium by the elastica interna. Buds composed of cells which could be considered endothelial were not observed during the course of this investigation. Pedunculated forms were never observed.

Anastomoses between arterial trunks were encountered occasionally within the depths of the gland. Figure 6 shows an anastomosis between two arterial trunks. Silver carbonate granules can be seen in the lumen of the anastomotic loop and the continuity of the lumen between the two trunks is clear. Anastomoses between smaller vessels, and between two branches of the same trunk shortly after their origin, have also been observed.

A noticeable feature in the arteries of the thyroid is the sudden change in the thickness of the coats and the caliber of the lumina of some of these vessels. Through a short length of vessel the caliber will be appreciably greater, and then it will return to its original size. Figure 5 shows such a case. In this preparation only the endothelial cement is impregnated. It will be noticed that there is no branching at the point of dilatation. Such dilatations are extremely common. Diminution in the caliber of the lumina of arteries for a short length in the absence of branching has also been observed, although with relatively less frequency. Abrupt changes in the thickness of the muscle coats were found (fig.

7). In this case the muscle coat of the branch is much heavier than in the parent trunk. Abrupt thinning of an arterial coat in the direction of the flow of blood has also been observed. This latter phenomenon is especially conspicuous in the transition from artery to capillary.

In organs other than the thyroid, the pre-capillary arteriole shows sparse and scattered muscle cells which become less numerous and finally disappear in the capillary (fig. 11). The transition from artery to capillary in the thyroid is commonly very abrupt, while a few instances of gradual transition have been observed. The muscle coat of the smallest arteries suddenly ceases and a bare capillary emerges without appreciable change in the caliber of the lumen (figs. 9 and 10).

In studying the smallest arteries in the thyroid, in thick sections impregnated with the method of Golgi, the muscular coats of the vessels were seen to cease abruptly, as described above. When the emergent capillaries were followed, after running for a short length as such, without passing through the capillary bed of a follicle, some were seen to increase appreciably in caliber; some to enter veins directly. The most probable interpretation that may be advanced is that these represent arterio-venous anastomoses. Figure 13 shows a direct communication with a fairly large venous channel and figure 12 shows the enlargement of a capillary into a vein. In injected glands relatively larger arterio-venous anastomoses have been observed (figs. 14 and 15), in which an artery communicates directly with a vein rather than through an intermediate capillary. In these the continuity of the lumina and the injected particles, as well as of the vessel walls can be seen. The smaller arterio-venous communications appear to be in a position to shunt off the capillary beds of only a few follicles. They are fairly numerous and are not difficult to find, whereas the larger ones are relatively infrequently encountered.

Although Wangensteen ('29) states that there are no valves in the veins of the thyroids of the human, in some of the dog thyroids examined valves were extremely numerous in the

larger veins. Figure 16 shows three found in the same animal (two in the same system of veins). Note the concentration of blood above the valves. The valves were only seen in serial preparations stained by the Cajal reduced silver nitrate methods in which they could be traced through several sections. In the sections cut with the freezing microtome the valves probably dropped out of the vessels during the manipulations incidental to their preparation.

DISCUSSION

The presence of arterial anastomoses within the thyroid has previously been demonstrated and needs no further comment. On the other hand, the presence of muscular cushions and of arterio-venous anastomoses has not been mentioned in the literature and may be of some physiological significance.

An objection to the interpretation of the dilatations of the capillaries as arterio-venous anastomoses lies in the possibility that the constricted arterial end may represent a contracted phase of the capillary, and the wider, venous end, a dilated phase. Waves of contraction have been observed and described in the capillaries in living animals. That the structures described here are not contraction waves, but are, in fact, anatomic enlargements in the caliber of the vessel and do represent arterio-venous anastomoses is strongly indicated by several features. The abrupt cessation of the muscular coat of the artery has been described as characteristic of arterio-venous anastomoses by Grant ('30). This is a constant feature of these structures in the thyroid. If they were waves of contraction, then one should in the course of observation on a great number of waves find some in which the distal end is contracted and the proximal end dilated. This was never observed in the capillary communications. Furthermore, the presence of arterio-venous anastomoses of a larger caliber, in which vessels could be specifically identified, has been noted, and, therefore, the presence of similar, smaller structures is to be expected. Granted that these communications are between arteries and venules and that the

connecting loop is only of the magnitude of a capillary, they must still be considered as arterio-venous anastomoses because of the shortness of the communicating loop and because they avoid the capillary bed of the follicles.

The normal presence of arterio-venous communications in the skin of the digits of humans and in the toes of birds has long been known (Berlinblau, 1875; Hoyer, 1877; Grosser, '02; v. Schumacher, '15; Heimberger, '25). Sandison ('28) observed arterio-venous anastomoses in the transparent chamber introduced into the ear of the living rabbit. Grant ('30) has also observed the constant presence of great numbers of such structures in the ear of the living rabbit. These experiments by Grant and those in a subsequent study (Grant, Bland, and Camp, '32) demonstrate that the arterio-venous anastomoses dilate on mechanical irritation, application of histamine, acetylcholine, and cold, and that they constrict upon stimulation of the cervical sympathetics and local application of epinephrin. The histological studies of the structures show that the walls of the arterial end of the anastomotic loops are thicker than usual in a vessel of the same caliber, both in the muscular and adventitial coats, and that this thickening is greatest at the point of junction of the artery and vein; further, that the muscle cells cease, or become sparse rather abruptly at these points. It is also stated that the lumen of the arterial end of the anastomotic loop is smaller than the venous end. (Compare Grant's ('30) figure 15 with figure 14 of this paper.) Grant, however, did not describe direct arterio-venous communications of the capillary type described here. In a later paper, Grant and Bland ('31) describe arterio-venous anastomoses in great number in the skin of the human hand and the foot of the hen and duck. The conclusion drawn from the experiments were that these structures act to regulate the flow of blood through the skin and are an important mechanism in the regulation of body temperature.

Grant and Viko ('29) and Wearn ('28) noted communications between the coronary arteries and the branches of the

Thebesian veins in the heart. The former observers believe that these communications are only of the magnitude of capillaries, whereas Wearn believed from injection experiments that the communications are larger than capillaries. The significance of such anastomoses lies in the maintenance of circulation in the heart through the Thebesian vessels after the lumina of the coronary arteries have been occluded by arterio-sclerotic processes. Nisimaru and Steggerda ('32), in studies on the blood vessels in the spleen, believe, on the basis of perfusion experiments, that there is evidence for some sort of direct communications between the arteries and veins in that organ.

It is obvious that arterio-venous anastomoses can act to restrict the blood supply of a capillary bed by shunting blood directly into the veins. Other than this, whatever the physiological significance of these structures in the skin, the heart, or the spleen may be, this cannot be applied to the thyroid gland. In view of Nonidez's ('31 a) statement that the ganglia in the thyroid are probably vasomotor and not secretory, and since the thyroid can function normally and without anatomical change when isolated from its nervous supply (Manley and Marine, '15 and '16), it seems probable that there is no secretory nervous control of the follicular epithelium. This suggests a possible function for the arterio-venous communications and the muscular cushions. Namely, that these structures can act to regulate the flow of blood to the capillary beds in the thyroid and so control the amount of hormone absorbed into the blood stream. If the secretion of the gland is not dependent on a nervous control, but continues to secrete at a regular rate, as the thyroid apparently does, then the amount of hormone absorbed into the blood stream will be proportional to the amount of blood flowing through the capillary bed of the organ, and can only be controlled by some mechanism which can influence the circulation through the capillary bed. If this assumption be correct, then a significant physiological function for the arterio-venous anastomoses and the muscular cushions becomes evident. While

there is no experimental evidence for such a suggestion, it seems justifiable, on the basis of Grant's experiments, to assume that the arterio-venous anastomoses in the thyroid can open and close upon proper stimulation, just as they may do in the rabbit's ear. They may shunt blood directly into the veins or force blood through the capillary beds and thus control the amount of hormone absorbed. In general, they can restrict the flow of blood so that, under ordinary conditions, only a small section of the gland is supplying hormone, and can open the entire capillary bed when there is a demand for more of the thyroid incretion.

If the muscular cushions are considered as structures assuming the rôle of arterial valves (of which they are very suggestive), they can complement the function of the arterio-venous communications by decreasing the caliber of the lumina of the arteries, thus restricting the blood supply of larger regions of the gland. Inasmuch as these cushions are composed of muscular cells, activity on their part must be assumed. In the light of our present knowledge, the only possible action of such structures should be to alter the diameter of the lumina of the vessels in which they are situated.

SUMMARY

1. The vessels within the thyroid glands from twenty dogs were studied. In the main, thick frozen sections ($45\ \mu$) were used. Some of the glands were injected with opaque material through the superior thyroid artery. Several series of sections were studied.

2. The gradual transition from artery to capillary is not commonly found. The transition is usually an abrupt one, the muscular coat ceasing suddenly and a bare capillary emerging.

3. Sudden changes in the thickness of the muscular coats and the lumina of the arteries were also found.

4. Anastomoses between arterial trunks were seen in the depths of the gland.

5. Valves were found to be numerous in the larger veins in the substance of the gland.

6. Cushions composed of smooth-muscle cells are described as occurring at the junctions of an arterial trunk and its branch. These cushions are formed by an extension back of the muscle coat of the branch into the wall of the trunk.

7. Arterio-venous anastomoses in the gland are described.

8. It is suggested that the function of the direct arterio-venous communications may lie in the regulation of blood going to the capillary beds of the follicles and in this manner control the absorption of thyroid hormone, and that the muscular cushions or buds can complement this function by varying the caliber of the lumina of the smaller arteries at their origin.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Sections stained by the Cajal silver nitrate, pyridine, and alcohol technique, unless otherwise specified. *circ.*, circular muscle cells; *cush.*, muscular cushions; *dil.*, dilatation in artery; *end.*, endothelial cell; *long.*, longitudinal muscle cells; *pig.*, pigment in arteries.

- 1 Muscular cushion. Vessel cut in cross section just below branch.
- 1 a Same as figure 1, but a little nearer the branch.
- 2 Muscular cushions at branch.
- 3 Muscular cushions at branch.
- 3 a Muscular cushions at two branches in the same trunk.
- 4 Muscular cushion cut in longitudinal section. Section marked off is shown under oil immersion in figure 4 a. Hematoxylin and eosin.
- 4 a Drawing made under oil immersion of section marked off in figure 4. Note the presence of both longitudinal and circular muscle cells and the difference between the longitudinal muscle cells and the endothelial cells.
- 5 Dilatation in artery. Injected silver ammonium hydroxide. Not counter-stained.
- 6 Anastomosis between two arterial trunks in the depths of the thyroid gland. Silver granules in the lumen.
- 7 Thickening in muscular wall of an arterial branch.

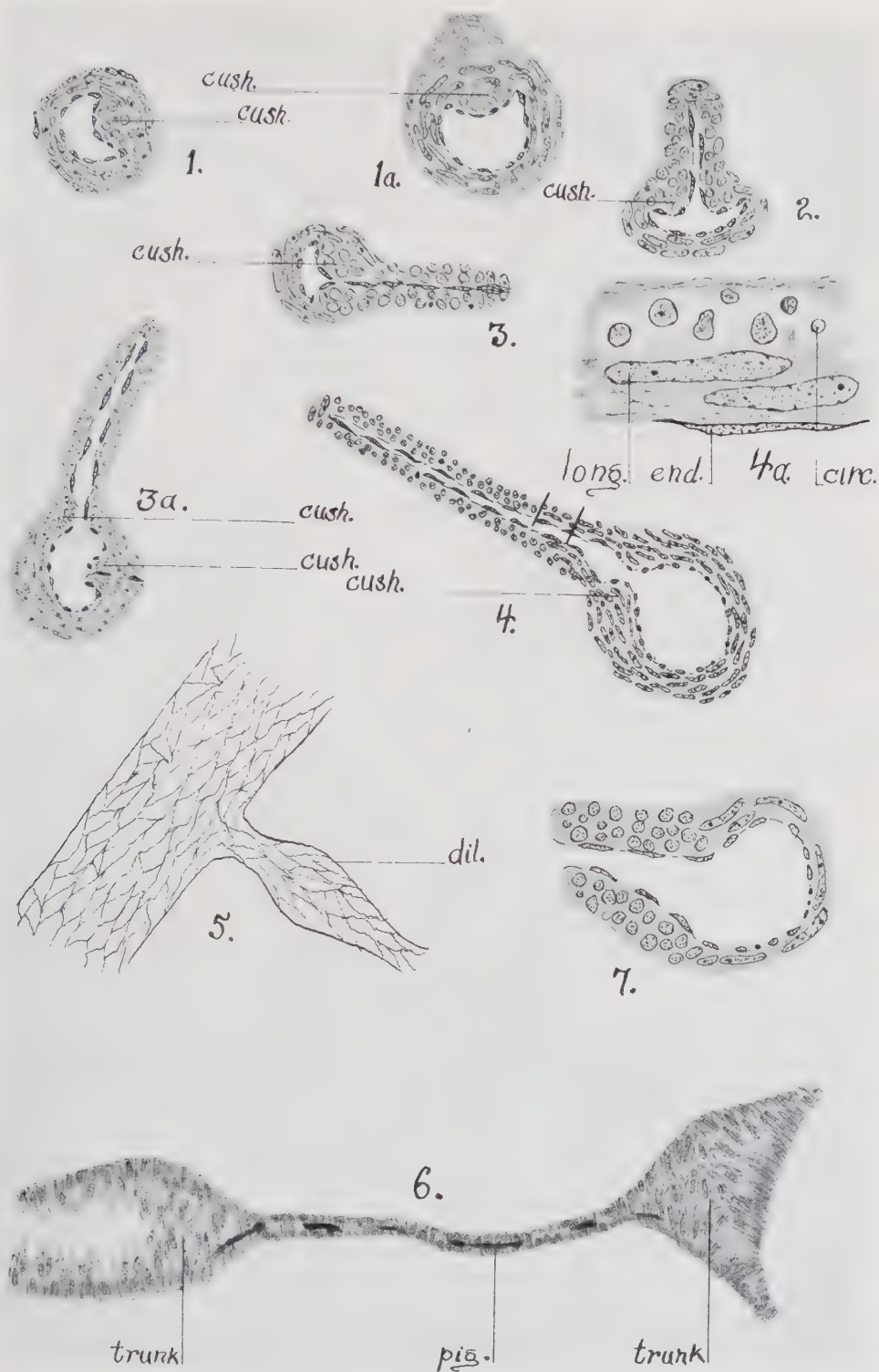
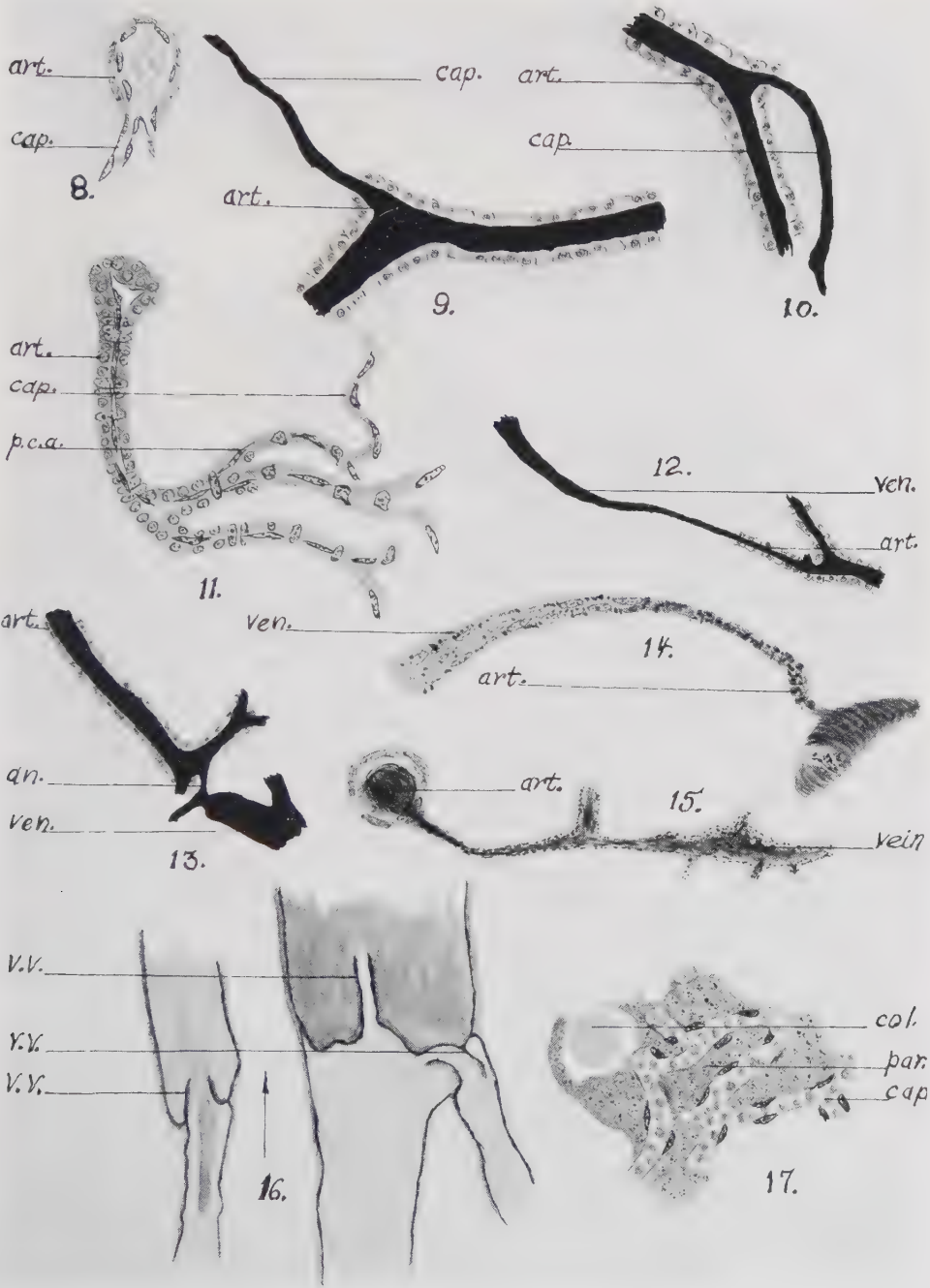


PLATE 2

EXPLANATION OF FIGURES

Sections stained by the Cajal silver nitrate, pyridine, and alcohol technique, unless otherwise specified. *an.*, anastomotic loop; *art.*, artery; *cap.*, capillary; *col.*, colloid; *par.*, follicular epithelium; *p.c.a.*, pre-capillary arteriole; *ven.*, venule; *v.v.*, valves of veins.

- 8 Two capillaries leaving artery with sudden loss of muscle.
- 9 Abrupt cessation of muscle coat in transition to capillary from artery. Golgi.
- 10 Abrupt cessation of muscle coat in transition to capillary from artery. Golgi.
- 11 Usual gradual transition from artery to capillary, showing scattered muscle cells on the pre-capillary arterioles. From tongue of rabbit. Sections cut 45 μ .
- 12 Capillary enlarging into a venule directly. Golgi.
- 13 Arterio-venous anastomosis. Golgi.
- 14 Arterio-venous anastomosis. Silver carbonate injected.
- 15 Arterio-venous anastomosis. Silver carbonate injected.
- 16 Valves in veins. Arrow points in direction of flow of blood. Cajal reduced silver nitrate.
- 17 Capillary bed on surface of follicle.



GROSS ASSIMILATION OF YOLK AND ALBUMEN IN THE DEVELOPMENT OF THE EGG OF GALLUS DOMESTICUS

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FOUR FIGURES

The most important constituents of the bird's egg are yolk and albumen. They furnish almost all the necessary food to the developing embryo throughout the entire period of incubation, and a considerable portion of yolk is used after the bird has been hatched. Therefore, it is generally assumed that during the incubation of the bird's egg the gross assimilation of the yolk and albumen is directly proportional to the growth of the embryo and to the exertation of energy by the developing embryo.

A review of the literature indicates that a few rather irregular observations on the amount of albumen in the developing egg have been made by Prout (1822), Prevost and Martin (1844), Curtis ('11), Bialaszewicz ('12), Komori ('16), Sendju ('25), Needham ('27), and Fangauf ('1928), and on the amount of yolk by Prevost and Martin (1844) and Sendju ('25). But the exact quantitative gross assimilation of the yolk and albumen throughout the incubation period has never been determined. The reason for this may possibly have been the extreme difficulty of separating the yolk from the albumen at certain periods of incubation, particularly when the yolk membrane is very tenuous and fragile.

Considering this issue to be of some importance to biologists, we tried to determine the gross assimilation of yolk and albumen at successive stages of embryonic development.

A method consisting in coagulation of yolk and albumen by heat prior to dissection (Romanoff, '32) was used. By employing this method, we could separate the various parts of

TABLE 1
Gross assimilation and changes in the moisture content of yolk and albumen

STAGE OF INCU- BATION	AVERAGE WEIGHT OF EGGS	AVERAGE WEIGHT OF YOLK	PERCENT- AGE OF DRY YOLK ¹	WEIGHT OF DRY YOLK ³	AVERAGE WEIGHT OF ALBUMEN	PERCENTAGE OF WET ALBUMEN ²	WEIGHT OF DRY ALBUMEN ⁴
<i>Days</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Grams</i>
0	61.2	19.26	52.84	10.18	34.63	11.40	3.95
1	60.2	19.72	51.56	10.17	32.91	11.19	3.68
2	60.4	18.66	50.59	9.44	33.86	12.64	4.28
3	61.3	21.15	50.53	10.84	30.86	14.50	4.47
4	59.4	20.68	49.95	10.33	21.71	18.83	4.09
5	60.6	22.06	46.90	10.35	17.49	23.79	4.15
6	61.0	20.10	45.98	9.24	14.79	31.18	4.61
7	59.4	19.97	43.95	8.78	12.17	35.23	4.29
8	61.5	20.61	46.45	9.57	12.48	40.11	5.01
9	62.2	21.43	44.67	9.57	11.65	37.10	4.32
10	60.8	19.81	45.00	8.91	11.16	37.14	4.14
11	62.5	17.80	47.90	8.53	11.10	42.02	4.66
12	61.5	20.93	45.74	9.57	10.36	44.09	4.57
13	60.6	19.41	48.90	9.49	8.99	38.60	3.47
14	63.1	18.95	42.50	8.05	6.76	39.20	2.65
15	62.5	16.17	48.77	7.87	5.93	38.29	2.27
16	62.3	17.48	52.60	9.19	2.29	38.85	0.89
17	62.3	14.24	52.16	7.30	0.49		
18	61.5	16.12	47.95	7.87	1.29		
19	62.1	15.18	54.96	8.34	0.03		
20		10.93	58.07	6.35	None		
Hatched							
1		7.28	54.99	3.27			
2		7.09	57.51	4.08			
3		5.34	56.49	3.02			
4		3.51	53.31	1.87			
5		3.50	52.56	1.84			
6		2.57	53.67	1.38			

¹ and ² Experimental data (Romanoff, '30).

³ and ⁴ Calculated.

an egg and determine their weights with precise accuracy, thus determining its quantitative utilization and interchange.

The experimental data here presented (table 1) were obtained from the eggs of a flock of White Leghorn hens (*Gallus*

domesticus). The eggs were carefully selected for uniform size and quality of egg shell. The incubation was carried on under standardized conditions (Romanoff, '30), with the temperature $38.0 \pm 0.2^{\circ}\text{C}$., the relative humidity 60.0 ± 1.0 per cent, and an ample supply of fresh air. At intervals of 24 hours ten eggs with normally developed embryos were dissected, and yolk and albumen were separately weighed.

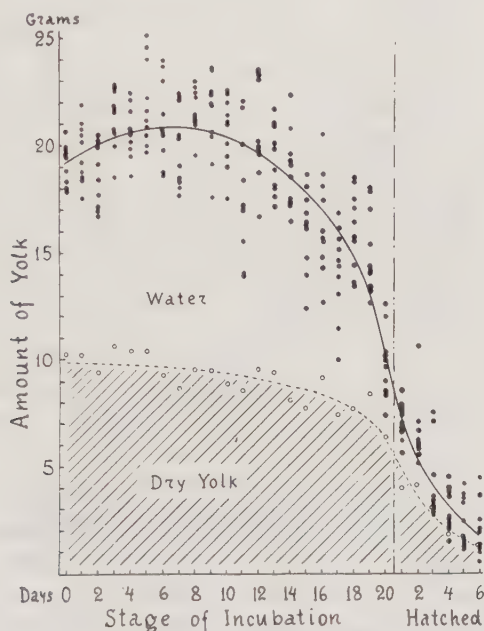


Fig. 1 Gross assimilation and changes in the moisture content of yolk.

The yolk, as shown in figure 1, noticeably gained in weight during the first week of incubation. After that time the curve of the weight turned downward, with a very steep decline near the end of incubation. A considerable amount of yolk (about 10 gm. together with yolk sac) was retained in the chick and was gradually assimilated within a week after hatching.

The curve of the amount of dry matter in yolk, calculated from our previous data (Romanoff, '30) is expressed in a

slowly descending line, which shows that yolk is being utilized from the beginning of incubation. Thus the gain in water content of yolk at that time may be attributed to the diffusion of water from the adjacent part of the egg.

The weight of albumen (fig. 2) in the developing egg decreases rapidly, with a sudden sharp drop from 34 gm. at the start to about 12 gm. at the seventh day of incubation.

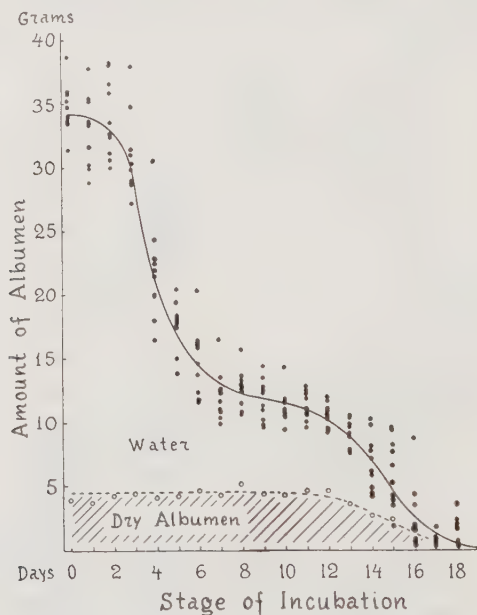


Fig. 2 Gross assimilation and changes in the moisture content of albumen.

Then the decrease slows up, but makes a second drop at about 12 days of incubation, with complete disappearance at the nineteenth day.

The amount of dry content of albumen seems to remain unchanged during the first 9 days of incubation. Then it begins to diminish and follows closely the trend of the curve of the wet weight. The curves of both wet and dry amounts of albumen strikingly demonstrate the apparent loss of water from albumen during the first few days of incubation.

The observations on individual eggs show that there is at all stages of incubation a considerable variation in the weight of yolk and albumen from one egg to another. This variation seems to exist irrespective of the size of the egg, but it is likely that it is the characteristic of the eggs of an individual hen.

Our previous observations (Romanoff, '29 a, '30) indicate that the loss in weight of fertilized eggs exposed to identical experimental conditions was very constant throughout the incubation period, and that the daily loss was on an average about 0.5 gm. It is of great interest and significance to note here that while there are such sudden and sharp changes in the water content of egg constituents, the loss in weight of the whole egg continues constant. This phenomenon may be explained by the influence of various physicochemical changes which go on within different parts of the developing egg, such as variations in the osmotic pressure (Bialaszewicz, '12), viscosity (Bellini, '07), total acidity (Aggazzotti, '13), hydrogen-ion concentration (Romanoff and Romanoff, '29), and perhaps several other factors.

To illustrate this point, figure 3 is intended to show the relative changes in the wet weight of the embryo, yolk, and albumen during the process of incubation. From this figure, as well as from table 1, it is evident that in the first part of incubation, only albumen loses its water. This loss or transformation of water may be accounted for by: 1) the absorption of water by yolk—this absorption presumably begins from the moment when the yolk comes into contact with albumen in the oviduct (Bialaszewicz, '12) and continues until the originally tenuous and fragile yolk membrane becomes tough and heavy (Byerly, '32); 2) the use of water in the formation of amniotic and allantoic fluids; 3) the water used for the developing embryo; and, 4) the loss of water through external evaporation, the rate of which depends largely upon the size of the egg, the individual structure of the eggshell, and the environmental conditions of incubation, such as humidity, temperature, movement of air, etc.

The changes in the dry weights of yolk and albumen (fig. 4) indicate that there is a gradual decrease in the total weight

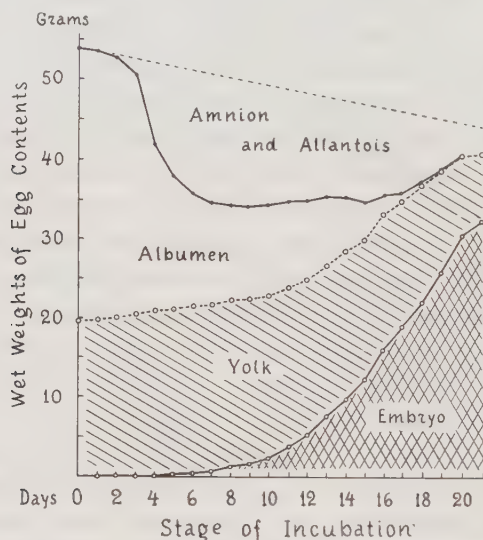


Fig. 3 Relative changes in the wet weight of various egg constituents. (Broken lines indicate the total decrease in the weight of egg contents. Ref. Romanoff, '30.)

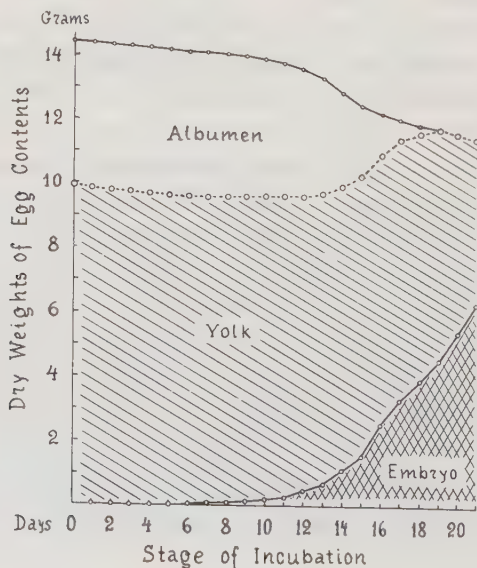


Fig. 4 Relative changes in the dry weight of various egg constituents.

of egg contents. This decrease corresponds to the katabolized amount of egg substances, the amount of which is directly proportional to the progressive development of the embryo.

CONCLUSIONS

The yolk and albumen of the fowl's egg undergo most profound changes in the course of embryonic development. Most of these changes involve transference of water from one part of the egg into another.

The water content of albumen rapidly decreases during the first part of incubation. At the expense of this water there is a noticeable swelling in the yolk; a large portion of water goes for the formation of embryonic membranes, some indirectly for the growth of embryo, and finally some of it is lost through external evaporation.

As the result of these modifications the yolk at first gains in its moisture content, then begins to decrease and after 2 weeks of incubation reaches its original value.

The dry substance of albumen, irrespective of its water content, is almost exclusively assimilated during the second half of incubation, until it completely disappears just before hatching.

The dry substance of yolk is gradually being assimilated by the developing embryo from the beginning of incubation, and the largest portion of it is used in the few days immediately before hatching. A considerable part of the yolk is used after the bird's exclusion from the shell.

The decrease of dry weight of yolk and albumen combined corresponds to the amount of katabolized egg substances, and it is directly proportional to the progressive development of the embryo.

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THE RESPONSE OF THE HEDONIC GLANDS OF THE
MALE TRITURUS VIRIDESCENS TO
HETEROPLASTIC IMPLANTS OF
ANTERIOR PITUITARY

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TWO PLATES (EIGHT FIGURES)

The hedonic glands, located on the sides of the head of the male *Triturus*, seem to play an important role in the mating behavior of the newts; their secretion serves to attract the female during courtship. The development and anatomy of these glands have been described by Hilton ('02), who found that they were rudimentary in the female, but in the male attained a certain level of development at the time of sexual maturity, and became greatly hypertrophied each year at the approach of the mating season.

Noble ('29) has described glands of similar function in the male plethodontid salamanders in which, however, the glands are not restricted to one location but are widely scattered over the dorsal surface, being abundant on the eyelids, temporal region, and especially the base of the tail. The relation of these hedonic glands to the sex hormones was investigated by Noble ('31), who removed the ovaries from adult *Desmognathus fuscus* females and implanted testes. Hedonic glands appeared in the skin of the base of the tail 62 to 220 days after implantation. They resembled young granular glands of the integument, but apparently arose independently of both these and the mucous glands.

Many recent papers indicate that the hypophysis-gonad-reproductive tract interrelationship, so strikingly demonstrated for mammals, prevails also in amphibians. These

are adequately summarized by Burns and Buyse ('31). Several studies have included observations on the secondary sexual characters, but no detailed study has been made of the response of the hedonic glands to the anterior-pituitary stimulation. However, Adams ('32) has observed the marked development of hind leg pads and tail fin following injections of a mammalian anterior-pituitary extract, phyone. In this paper the response to anterior-pituitary stimulation of the hedonic glands of normal, unilaterally and bilaterally castrated males is described.

MATERIAL AND METHODS

Sexually mature aquatic newts were used. Individuals were selected in which the external secondary sexual characters, cloaca, tail crest, femoral corrugations, and toe pads were undeveloped. In every case, previous to the anterior-pituitary treatment, the hedonic glands were removed from one side and studied histologically, to determine their state of activity. Thus each animal served as its own control, the assumption being made that the physiological state of the glands of the two sides at any one time would be the same.

In addition, in some animals one testis and in others both testes were removed prior to the treatment. At the close of the experiments, the remaining set of hedonic glands, the testes, and, in a few instances, the thyroid gland were fixed and sectioned. Thus there were available for study and comparison hedonic glands, both before and after treatment, from the same individuals of which some were normal, some unilaterally castrated, and others bilaterally castrated. This series also made possible observations on the responses of the thyroid and testis to anterior-pituitary stimulation.

Three different types of anterior-pituitary stimulation were attempted. One series of animals was treated by intraperitoneal injections of antuitrin prepared by Parke, Davis & Co. A second series received intraperitoneal injections of an extract of the whole pituitary gland of the sheep, prepared and distributed for research purposes by the above named con-

cern. A third series received intraperitoneal implantations of fresh anterior lobe taken from the frog, *Rana pipiens*. Antuitrin, injected intraperitoneally, proved toxic, causing the accumulation of large quantities of fluid in the abdominal cavity; the animals died before any positive results were obtained. The whole sheep gland extract, injected in 0.10 cc. amounts daily for a period of 15 to 20 days, yielded positive results, but seemed slightly toxic with the dosage employed. Moreover, the presence of the melanophore-expanding principle caused the animals to become very dark, thereby hindering observations on the early stages of pigmentation of the femoral ridges and toe tips, which were found to be good indicators of the response of the animals to anterior-pituitary stimulation.

The most satisfactory results were obtained with implantations of fresh, anuran, anterior lobe, the animals remaining normal in every respect. In this series each animal received a total of sixteen anterior lobes, implanted two at a time, at varying intervals of 2, 3, and 4 days. Usually a well-defined response was observed after six lobes had been used, but the maximum development of the external secondary sexual characters was obtained only when a total of sixteen had been implanted. The following description is based on the responses obtained following anuran anterior-lobe implants. Positive results were obtained also with the extract of whole mammalian gland, but it was felt that in this series the changes obtained might be modified to some extent by the presence of substances from the other divisions of the hypophysis.

RESULTS

a. Response of normal animals

Little direct evidence of the reaction of the hedonic glands to anterior-pituitary stimulation could be obtained by inspection of the living animal. The integument of the side of the head was not greatly modified. The glandular area became slightly elevated above the surrounding integument,

and the pits into which the glands empty became larger and deeper. Other external features, however, showed striking changes. The tail crest became prominent, the cloaca increased in size, the femoral ridges were thickened and deeply pigmented, and the tips of the digits of the hind feet became black (Adams, '32). Thus the progressive acquisition of the typical nuptial apparel (figs. 1 and 2) served as an indicator of the response of the entire animal to the treatment.

The activity of the tubules of the hedonic glands may vary considerably in different animals, and in many instances the individual tubules of a glandular area may show varying degrees of activity (figs. 3, 5, 7). In the resting stage the secretory epithelium is composed of tall columnar cells with elongated nuclei. The cells extend toward the lumen and sometimes almost meet at the center. This is usually the case in light-colored animals which have just completed their terrestrial stage. In other animals the tubules are slightly distended with colloid which may have been retained from prior glandular activity.

However, these variations in tubular activity of untreated animals are not sufficiently great to hinder the recognition of the response to anterior-pituitary stimulation. In animals which have received a maximum dosage of sixteen implants the hedonic glands are strikingly hypertrophied. Their lumina are widely distended and contain a colloidal secretion. The epithelial cells are low cuboidal or even squamous (fig. 4). Cross sections of such tubules resemble sections of colloidal vesicles of the thyroid, and the surface of the colloid in the hedonic gland frequently is marked by vacuoles similar to those characterizing thyroid colloid.

b. Response of unilaterally castrated animals

The removal of one testis prior to treatment does not appear to modify the stimulating effect of the anterior lobe (fig. 6). It seemed at first that the reaction of the gland was slightly reduced, but later studies showed that the induced glandular hypertrophy obtained after unilateral castration

was equal in many instances to that found in treated, normal animals. The response of individual tubules of the same glandular area is not always constant and it is consequently difficult to make significant quantitative observations.

c. Response of bilaterally castrated animals

When both testes are removed, the hedonic glands fail to hypertrophy following treatment with anterior-pituitary implants (figs. 7 and 8). The tail fin, however, shows the same degree of enlargement as noted in normal and unilaterally castrated animals, but the hypertrophy of the cloaca and the blackening of the femoral corrugations and toe tips do not occur. The disproportion of tail and cloaca give the treated, castrated males an odd appearance.

d. Changes in the testis

The testes of all animals were studied histologically. Only in unilaterally castrated animals was it possible to measure the effects of anterior-pituitary implants on the testes, since in these instances one gland was obtained before treatment and the other after treatment. The study of the testes of normal animals after treatment and of bilaterally castrated animals yielded some supplementary data besides indicating that the two testes of any one individual were in comparable stages of spermatogenic activity.

The testis of *Triturus* is a multiple one, consisting of more or less separate lobes. The complex structure depends on the operation of two factors: a slow caudocephalic spermatogenic wave and a delayed regeneration of the emptied or degenerated lobules (Humphrey, '26). The testes studied were composed of either 2 or 3 lobes. In all untreated animals the most caudal lobe was filled with fully differentiated spermatozoa, while the more cephalic lobes were filled with less mature elements which gave no evidence of mitotic activity.

After treatment the caudal lobes of all testes examined were found to be either empty or in a very early regenerative

stage, while in the immature, cephalic lobes large areas of cells were observed undergoing mitotic division. There was also a definite increase in the size of the testes. They were not weighed, but estimates based on serial sections indicated an increase in size of 200 to 400 per cent. No spermatophore deposition was observed in treated animals. However, the wolffian ducts and seminal vesicles were greatly hypertrophied, and it was concluded that the sperm from the emptied lobes were stored in the vesicles.

e. Changes in the thyroid gland

Only incidental observations were made on the thyroid glands. The thyroid follicles were collapsed and contained very little colloid. The cells of the follicular epithelium were tall columnar in form and contained large quantities of stainable colloid distributed throughout the cytoplasm as small granules or globules. Vacuoles with unstainable colloid were also present. In almost every respect the picture presented by the glands of the adult *Triturus* was in agreement with Grant's ('30, '31) description of the thyroid of *Necturus* and larval urodeles following anterior-pituitary stimulation.

DISCUSSION AND SUMMARY

Full development of all secondary sexual characters of adult male newts may be induced out of season by anterior-pituitary stimulation. Adams ('32) demonstrated that the development of the femoral corrugations was dependent upon the presence of the testis, whereas the increase in size of the tail fin, although a usual accompaniment of accelerated gonadal activity, was obtained in castrated males. The tail crest, accordingly, cannot be regarded as a true secondary sexual character under the influence of the testicular hormone, but is a growth phenomenon controlled directly by the growth hormone of the anterior-pituitary.

This study confirms the observations of Adams and extends the information to include the hedonic glands. These glands were found to respond in the manner characteristic of

secondary sexual characters, undergoing great hypertrophy in treated, normal, and unilaterally castrated males and failing to respond in bilaterally castrated males. Unlike the tail fin, they are not directly affected by the growth principle, but require the presence of the testes. It is possible that the growth hormone may be involved in effecting the final result, but the pituitary principle controlling gonadal development is essential for the response.

The presence of normal, testicular tissue is not sufficient to maintain the hedonic glands at a high level of activity. In all the untreated animals in which the hedonic glands were relatively inactive the caudal lobes of the testes uniformly contained mature sperm while the other lobes contained cells in varying stages of spermatogenesis. Hypertrophy of the hedonic glands appears to be dependent on the attainment by the gonad of a relatively high level of activity.

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PLATE 1

EXPLANATION OF FIGURES

1 Side view of a unilaterally castrated animal after sixteen anterior-pituitary implants, showing tail fin, femoral corrugations, and blackened tips of the digits of the hind limbs.

2 Ventral view of the same animal, showing the conspicuous blackened femoral corrugations and enlarged cloaca.

3 Cross section of the pit and a few tubules of the hedonic gland of a normal animal. $\times 100$.

4 Cross section of tubules of the hedonic gland from the opposite side of the same animal, showing the extensive hypertrophy and accumulated colloid following implants of anterior pituitary.

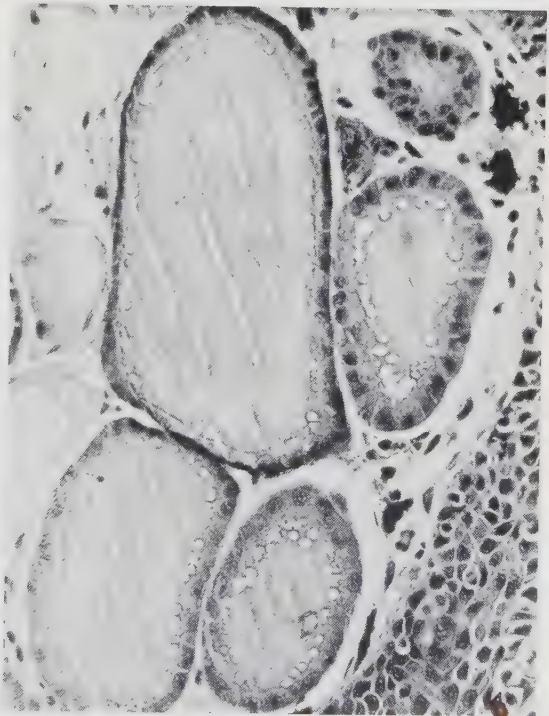
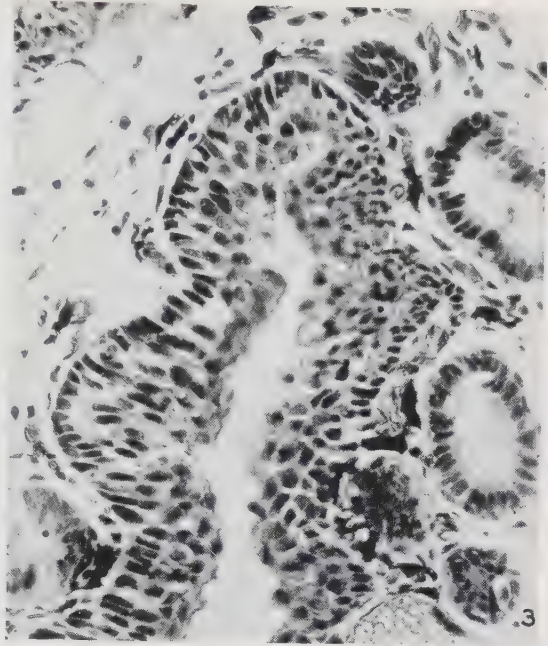


PLATE 2

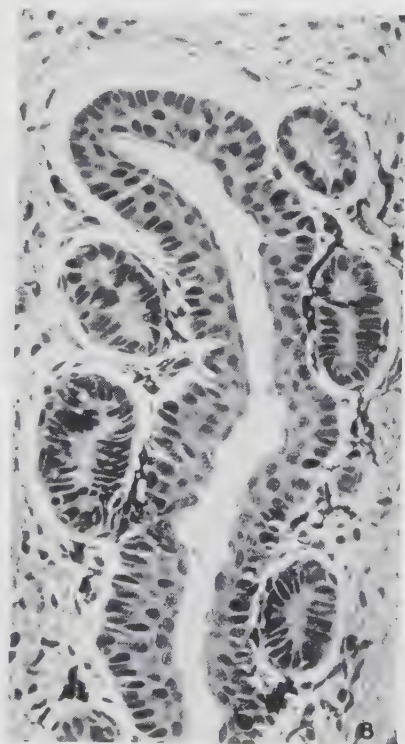
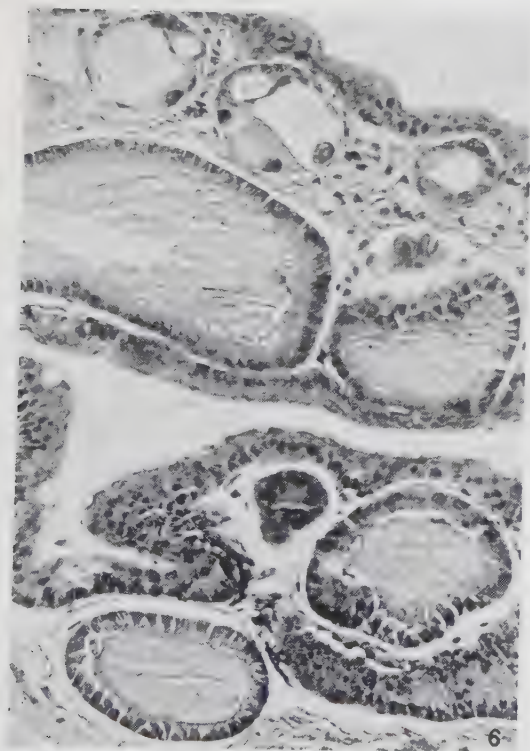
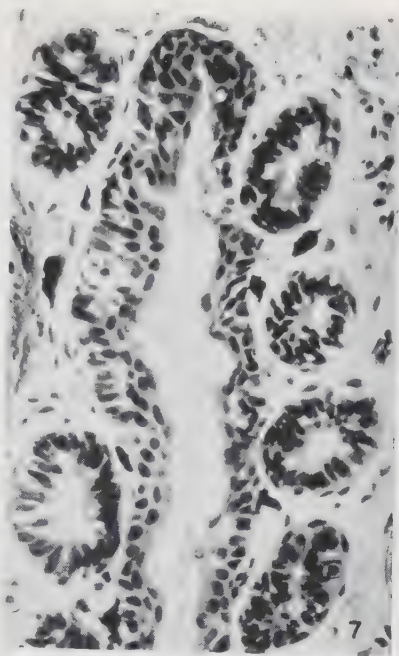
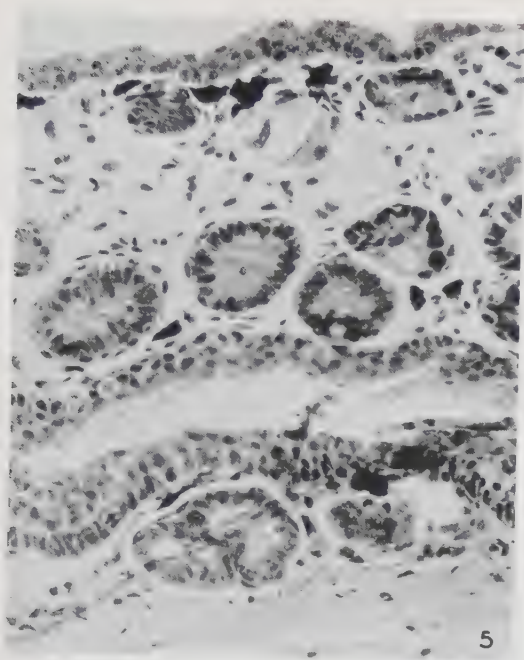
EXPLANATION OF FIGURES

5 Section of the pit and tubules of the hedonic gland of a normal animal at the time one testis was removed. $\times 100$.

6 Section of the pit and tubules of the hedonic gland from the opposite side of the animal following anterior-pituitary treatment. The same hypertrophy and accumulation of colloid was obtained as in figure 4. $\times 100$.

7 Section of the pit and tubules of the hedonic glands of an untreated animal at the time both testes were removed. $\times 100$.

8 Section of the glandular area from the opposite side of the same animal after anterior-pituitary treatment. Note the absence of any hypertrophy or accumulation of colloid. $\times 100$.



THE ATTACHMENTS OF THE DURA MATER OVER THE BASE OF THE SKULL¹

A. EARL WALKER

ONE FIGURE

To the surgeon operating in the intracranial cavity the attachments of the dura mater to the cranium have considerable practical importance, yet detailed descriptions of them are difficult to find. For this reason the present investigation was made at a series of necropsies. The ages of the subjects varied and they had died from a variety of causes. However, from the anatomical standpoint these factors seemed not to be very important. The method used was simply to strip the membrane from the inner surface of the skull, carefully noting the points to which it was attached and the structures which were involved in these attachments. The findings are not entirely constant, but the essential variations are few.

THE ATTACHMENT TO THE CONVEXITY

The strongest attachment to the cranium on the convexity is in the midline. Here the superior sagittal sinus is firmly attached by fibrous processes of the outer dural layer to the midline of the skull. Less firmly it is attached to the lambdoidal and coronal sutures. Between these points it is easily stripped with only scattered unimportant attachments principally along the branches of the middle meningeal artery.

¹ From the Division of Neurology and Neurosurgery, University Clinics, University of Chicago. This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research at The University of Chicago.

THE ATTACHMENT TO THE BASE

The anterior fossa

The beginning of the superior sagittal sinus is frequently a small vein from the nose which passes up through the foramen caecum. Around this vein the dura mater is firmly attached. Posteriorly, in the midline of the base, the membrane is firmly attached to the crista galli of the ethmoid bone, on either side of which lie the grooves for the olfactory bulbs. In this region are the many small foramina through which pass the olfactory nerves. Around them and also around the ethmoidal foramina, which communicate with the orbit, the dura mater is firmly attached. Inconstantly, lateral to the olfactory grooves, small branches of the meningeal vessels enter the inner table of the skull. In the posterior part of the anterior fossa there are a number of attachments. On either side of the midline the optic nerves enter the orbit through the optic foramina and around these nerves the dura mater is prolonged forward to the orbital wall. Between the nerves and from the anterior clinoid processes the membrane can be stripped, although usually with some difficulty. The sheet which forms a thin capsule for the pituitary body is usually easily stripped from the hypophysial fossa, but is frequently so thin on the posterior part of the hypophysis that it cannot be followed to the posterior clinoid processes. To the most lateral tips of the lesser wings of the sphenoid bone the dura mater is attached by folds around the anterior branch of the middle meningeal artery which frequently tunnels the bone at this point. Extending medially along the wing, it is less firmly attached along the sphenoparietal sinus. Stretching across the midline in the posterior part of the floor of the anterior fossa is a strong band about 2 cm. in width which gains attachment to the jugae cerebrales in the middle part of each anterior fossa.

The middle fossa

Stripping the dura mater from the lateral to the medial side of the middle fossa, we first often note firm attachments to the jugae cerebrales between the digitations on the floor of the fossa. Around the structures entering the superior orbital fissure—the oculomotor, trochlear, ophthalmic division of the trigeminal, abducens nerves with the ophthalmic veins, sympathetic branches to the ciliary ganglion, and small orbital branches of the middle meningeal artery—the membrane forms a sheath which runs into the orbital cavity, merging with the periosteum of the bony orbit.

Along the medial side of the middle fossa the dura mater forms a thick mass in which lie the cavernous sinus, the abducens nerve, the maxillary and ophthalmic divisions of the trigeminal nerve, and the internal carotid artery. Around the maxillary nerve it forms a sheath continued into the foramen rotundum; similarly it is carried into the foramen ovale around the roots of the mandibular nerve, and the accessory meningeal artery. As these nerves and vessels pierce the dura mater, sheaths follow them for a short distance. From the anterior surface of the petrous pyramid it can be readily stripped as far as the foramen lacerum, where it is firmly attached to the vessels and nerves passing through.

The posterior fossa

To the midline of the occipital bone, above the transverse sulcus, the dura mater is irregularly attached along the line of the sagittal sinus. The attachment of the lateral sinuses to the transverse sulcus is not very firm. The attachments to the inferior occipital fossa are very feeble and easily broken. It is better attached to the ridge from the internal occipital protuberance to the foramen magnum, along the posterior border of the foramen magnum and around its sides to the canales hypoglossi.

In the anterior part of the posterior fossa the dura mater is rather firmly attached to the posterior clinoidal processes.

Backward from them along the clivus it is attached by dense fibrous tissue and is separated only with difficulty. In the upper part of the posterior fossa, on either side, the dura mater accompanies the acoustic and facial nerves as they sink into the internal acoustic meatus, and is rather firmly attached to the superior petrous sulcus for a centimeter or more along the superior margin of the temporal bone behind the acoustic meatus. Immediately below this region it is attached to the sulcus for the sigmoid portion of the transverse sinus and more anteriorly follows around the ninth, tenth, and eleventh cranial nerves as they enter the jugular canal. The dura mater is further attached to the sulcus for the transverse sinus where the posterior temporal diploic vein passes through the mastoid foramen to empty into the transverse sinus.

The attachments over the base of the cranium described in the foregoing paragraphs are illustrated in the accompanying diagram (fig. 1).

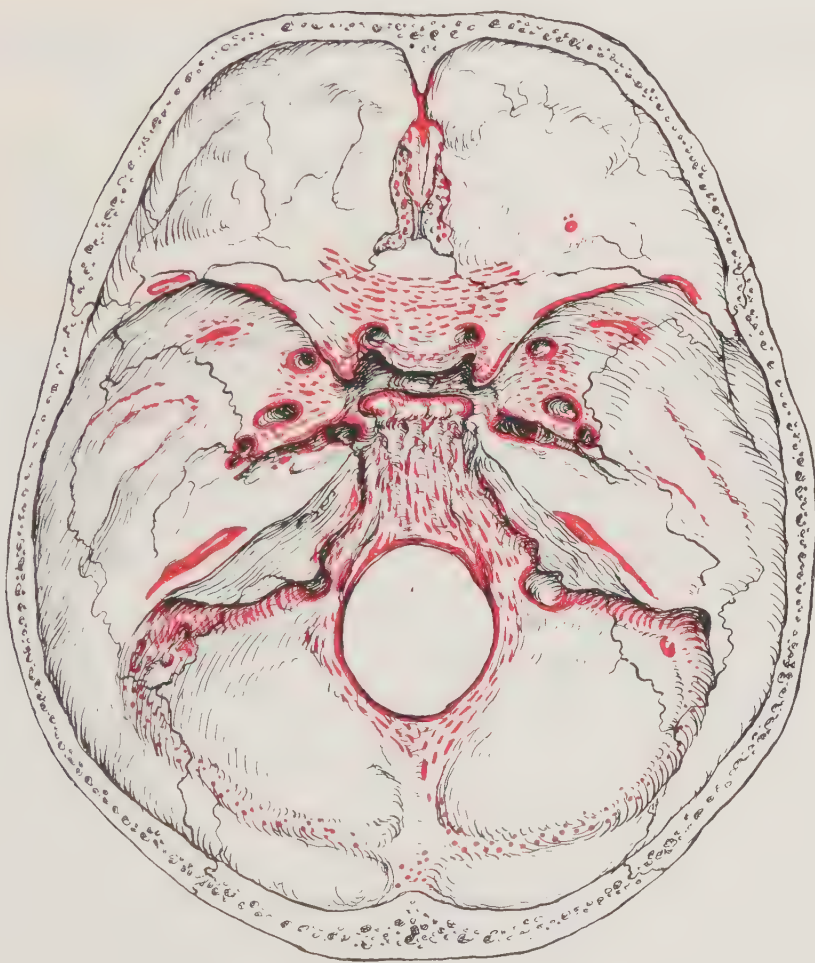


Figure 1

THE NATURE OF THE THORACIC AND ABDOMINAL DISTRIBUTION OF THE VAGUS NERVES¹

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TWO PLATES

INTRODUCTION

The vagus nerves, because of their extensive distribution and because of certain features involving the embryological development of several organs, such as the aortic arches, stomach, and gonads, have always been of a problematical nature to the anatomist. Though investigators in this field, from the eighteenth century to the present day, have successfully unraveled the more intimate details concerning the peripheral course of the vagi, not all of the authors and editors of contemporary textbooks have availed themselves of the data on hand, with the result that many students, who do not make a special study of the subject, are deprived of facts which are of major importance for a correct understanding of visceral innervation.

THE INTERCHANGE OF FIBERS BETWEEN THE VAGUS NERVES IN THE THORAX

It is a commonly known fact that, caudal to the roots of the lungs, the relative right and left position of the vagi are no longer retained. This change in position is caused by the rotation of the stomach; and concerning it Arey ('30) writes:

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The author wishes to express his appreciation to Dr. Eduard Uhlenhuth for his most helpful guidance and criticism in the execution of the work reported.

The original right and left surfaces, carrying the corresponding vagus nerves, necessarily become dorsal and ventral; this circumstance logically explains the otherwise puzzling asymmetry of the vagi at this level.

In concordance with the above view, Robinson ('30) like several authors and editors of textbooks, states that the right vagus—

emerges from the Plexus [posterior pulmonary] usually as a single trunk which runs downwards and medially, in the posterior mediastinum, to the oesophagus. On the oesophagus it breaks up into branches which unite with branches of the left vagus to form the oesophageal plexus. At the lower end of the thorax the right vagus again becomes a single trunk; it passes to the posterior aspect of the oesophagus and enters the abdomen through the oesophageal orifice of the diaphragm.

About the left vagus Robinson writes:

At the lower border of the root of the left lung it emerges from the plexus as two cords which descend, into the posterior mediastinum, to the oesophagus, where they unite with branches of the right vagus to form the oesophageal plexus. At the lower end of the thorax the left vagus again becomes a single trunk, which passes through the oesophageal orifice of the diaphragm on the anterior aspect of the oesophagus.

The descriptions quoted above, though in conformity with the embryological facts, do not agree with certain data which are based on the findings of Swan (1834), Kollmann (1860), Wertheimer ('01), McCrea ('25), Brauecker ('27), Uchida ('28), and Teitelbaum and Uhlenhuth ('32). The work of the above-mentioned investigators, based on both dissections and degeneration experiments, has shown that each vagus nerve as it continues caudal to the pulmonary root with which it is associated, breaks up into several long nerve fibers which incline medially as they descend along the oesophagus. The long medial rami from the right vagus pass both ventral and dorsal to the oesophagus, as do the corresponding rami of the left vagus nerve. Anastomoses take place on the respective surfaces of the oesophagus, forming the anterior and pos-

terior oesophageal plexuses. Very often, however, the long medial rami do not lose their individuality as they course through these plexuses, and on each surface of the oesophagus, just above the diaphragm, they can be seen uniting into two distinct cords which descend into the abdomen. Of these cords Greving ('31) says that they should henceforth be designated as chordae oesophageae, or, according to Brauecker, 'Chorda Anterior and Posterior,' since, as a result of an extreme interchange of fibers between the vagus branches coursing on the ventral and dorsal aspects of the oesophagus, differentiation into a right and a left vagus is no longer possible. With the above before us for comparative purposes, it is very evident in what respect Robinson's description, which maintains that the right and left vagi emerge from the anterior and posterior oesophageal plexuses, respectively, differs from the more acceptable one of Greving. Robinson's description is misleading when one uses it as a basis for determining the innervation of the abdominal viscera, for it does not permit one to understand the bilateral distribution of each vagus in the abdomen. Even in the thorax each vagus has a bilateral distribution with relation to the lungs, as determined physiologically by Weber; and as Iwama ('25), and Larsell and Mason ('31) have demonstrated in degeneration experiments.

THE ABDOMINAL DISTRIBUTION OF THE POSTERIOR VAGUS CORD

Kollmann ('60) reviews the literature on the distribution of the posterior vagus cord in the abdomen from Haller (1762) to Sappey (1852). That the early investigators mentioned by Kollmann offered descriptions which differed to some extent is only natural. One can readily understand the reason for these diversities if one is acquainted with the intricate nature of the autonomic plexuses in the region concerned. In addition, the individual variations in the more detailed ramifications of the vagus branches to be found in each body undoubtedly explain many of the differences in opinion which existed at that time. Though the general pattern of

distribution of the vagus fibers in the abdomen is more or less constant, as shall be pointed out, individual variations do exist, and they are very evident in the two dissections illustrated in plates 1 and 2.

If all of the contributions up to Kollmann's time are considered, branches were then known to pass from the posterior vagus cord to both the right and left coeliac ganglia, the splenic plexus, the liver, pancreas, duodenum, gall bladder, stomach, renal plexuses, left suprarenal gland, diaphragm, aorta and its branches, and to the superior mesenteric plexus. Bourgerie (1844) was the first to describe the contributions to the suprarenal gland and diaphragm. The superior mesenteric branches were disputed by several. Bourgerie summed up the entire matter with the statement that all the viscera received vagus fibers, partly direct, and partly through the union with sympathetic nerves. Despite the many contributions by independent investigators, Kollmann claims that the descriptions in the texts of his time were very scanty. To a lesser degree, this is also true for a number of the modern texts of anatomy.

MATERIAL AND METHOD

The material used for the dissections were newborn male infants which had died at birth. They were embalmed; and kept in 75 per cent alcohol during the period of dissection. The bodies were decapitated and dismembered, and the ventral walls of the thorax and abdomen removed; as were the lungs, and the intestinal tract caudal to the duodenum. The tail of the pancreas and the splenic vessels were resected as illustrated in plates 1 and 2. All the dissecting was carried out with the aid of the binocular dissecting microscope at a magnification of about 13 diameters. The illustrations are free-hand drawings made to scale. For this purpose the diameter of the field of vision was arbitrarily assumed to be 2.5 cm. The end result was a picture large enough to show the more minute details of the dissection. The drawing and dissecting were carried out simultaneously; and the progress

of both were closely followed by Dr. Eduard Uhlenhuth, whose inspiring interest and meticulous criticism have been indispensable factors in the execution of the work reported.

The anterior and posterior coeliac plexuses

In dissecting the coeliac plexus on the left side, it was observed that the nature of this plexus was such as to warrant a differentiation of its constituent nervous elements into two distinct layers, an anterior and a posterior. The layer which lies anteriorly consists of a plexus which is formed by contributions from sympathetic as well as vagus fibers. This plexiform layer shall be designated as the 'anterior coeliac plexus.' In plate 1 the 'anterior coeliac plexus' (*v.c.p.*) is shown in detail in its position ventral to the left coeliac ganglion and between the resected pancreas (*p*) and the left adrenal gland (*ad.*). The tail of the pancreas normally lies ventral to this plexus. In plate 2 both the 'anterior and posterior coeliac plexuses' are dissected in detail. The anterior plexus is not as intricate as in plate 1, however.

Dorsal to the 'anterior coeliac plexus' lies a layer which consists of the left coeliac ganglion and the branches radiating from it. This layer, which shall be referred to as the 'posterior coeliac plexus,' has been dissected in detail in the specimen illustrated in plate 2 only. These two divisions of the coeliac plexus are joined by anastomoses. Whether a similar condition also exists on the right side cannot be decided at present.

The general pattern of the abdominal distribution of the posterior vagus cord

From an inspection of the plates it becomes obvious that the posterior vagus cord and its branches are arranged in a definite pattern. This same pattern was also found in several other dissections not illustrated here.

The posterior vagus cord (*D.V.C.*) enters the abdomen as a single (pl. 1) or double nerve trunk (pl. 2). McCrea ('25)

states that the number of trunks passing through the oesophageal orifice may be single or multiple on either side of the oesophagus. Teitelbaum and Uhlenhuth ('32) illustrate a double posterior vagus cord in an infant.

On the basis of their origin, the branches of the posterior vagus cord can be differentiated into three groups: 1) anterior branches; 2) right branches, or those arising from the right side; 3) terminal branches.

1. The anterior branches (*V.D.V.C.*, pls. 1 and 2) arise from the anterior aspect of the posterior vagus cord, and on the left side of the body they either pass to the coeliac ganglion or else they anastomose with the sympathetic fibers of the 'anterior coeliac plexus.'

2. The right branches (*Di.V.G.*, pls. 1 and 2) make up the gastric nerves which arise from the posterior vagus cord. These gastric nerves also send anastomosing branches to the anterior coeliac plexus (pl. 1).

3. The terminal branches (*T.D.V.C.*, pl. 2) are the direct continuations of the major portion of the posterior vagus cord. These either enter the left coeliac ganglion as in plate 1. in which the terminal fibers form a single thick trunk; or they pass to both coeliac ganglia, as in plate 2. In the latter plate, the right one of the two trunks forming the posterior vagus cord disappears behind the splenic artery (*s.a.*) and the pancreas (*p.*) to pass to the right side. The terminal branches of the other posterior trunk in plate 2 enter the left coeliac ganglion, but before entering it they give off several direct vagus branches which join the anterior coeliac plexus.

Kollmann (1860) refers to other investigators who claim that the posterior vagus cord may pass to the right coeliac ganglion. Hirt ('24) describes the posterior vagus cord in man as going to both coeliac ganglia, as does McCrea ('25). Whether the terminal branches of the posterior vagus cord go to one, usually the left, or to both coeliac ganglia, quite likely depends on whether the posterior cord is single or multiple. If single, it cannot very well go to both coeliac ganglia, unless

they lie in juxtaposition. But even if the right ganglion receives no vagus fibers directly, it most likely receives vagus contributions from the left ganglion for distribution to the viscera on the right side.

That the left coeliac ganglion also receives contributions from the anterior vagus cord was stressed by Uchida ('28). I have been able to confirm this branch of the ventral vagus cord in an adult dissection not illustrated here.

The 'direct and indirect vagus branches' of the posterior vagus cord

Taking the course rather than the origin of the branches of the posterior vagus cord as a distinguishing characteristic, it is possible to differentiate them into two groups, 1) indirect and, 2) direct vagus branches.

The 'indirect vagus branches' are those which pass through the coeliac ganglia before reaching their termination in the viscera. Since they lose their identity in the coeliac ganglia, they cannot be followed beyond the latter by gross dissection. 'Indirect vagus branches' consist of those terminal and ventral branches which enter the coeliac ganglia.

The 'direct vagus branches' of the posterior vagus cord consist of the anterior vagus branches, and of the offshoots of the terminal and right branches which do not pass through the coeliac ganglia. They are distributed to the various abdominal organs, either directly or through the 'anterior coeliac plexus.'

It is the prime purpose of this paper to stress the distribution of the 'direct vagus branches' on the left side of the body. On the right side, though they usually lose their identity in the coeliac plexus, the direct fibers can often be followed, especially to the hepatic plexus. It must be kept in mind that the 'direct vagus branches' do not necessarily consist of vagus fibers only; for anastomoses with sympathetic fibers take place in both the thorax and abdomen, therefore an absolute distinction between the two kinds of fibers is impossible in either gross or microscopic dissections.

The aorta. That vagus fibers are distributed to the aortic plexus and the plexuses which follow the branches of the aorta as mentioned in Kollmann's review is a natural deduction. The aortic plexus inferior to the renal vessels receives direct vagus fibers (*D.V.A.*) as is evident in plate 2. The larger of the two direct vagus branches to the aorta was followed almost as far down as the aortic bifurcation.

In the light of this observation, it is interesting to note that Kuntz ('29) is inclined to doubt the correctness of the conventional opinion which maintains that the vagus does not provide fibers to the pelvic organs. He states that,

It is highly probable, however, that the ganglion cells in the lower third of the ureter are incorporated in vagus efferent chains,

also—

The descending colon and upper part of the rectum are supplied by nerves which arise from the inferior mesenteric plexus. This plexus is a derivative of the aortic plexus, through which it also receives vagus fibers from the coeliac plexus. Therefore, the nerves supplying the descending colon, at least in man, include both vagus and sympathetic fibers.

Suprarenal glands. That the left suprarenal gland receives direct vagus fibers was, as has already been mentioned, determined just prior to the appearance of Kollmann's work. Kollmann (1860) mentions such fibers, as does McCrea ('25). Uchida ('28) describes a direct vagus branch to the left suprarenal gland from the anterior vagus cord of the human adult. He found this branch consistently. I have been able to follow such a branch in an adult cadaver, but its sympathetic anastomoses were of such a nature as to cast some doubt on its ultimate termination. Uchida could find no direct branch on the right side. My efforts to dissect direct vagus fibers from the posterior vagus cord to the right suprarenal gland were not successful. The vagus fibers lose themselves in the coeliac plexus. The right suprarenal gland, the right kidney, and the pancreas, according to Kollmann, receive vagus fibers through the right coeliac ganglion.

Recently, Hoshi ('27) studied the innervation of the suprarenal gland in the rabbit by means of degeneration experiments. Though he was able to demonstrate the disappearance of the nerves in the medulla after splanchnic nerve section, he found no changes from the normal after vagus section. Also Kure, Wada, and Okinaka ('31) claim that the vagus seems to play no significant role in the innervation of the suprarenal gland.

In plate 1 the only possibility of direct vagus fibers to the left suprarenal gland is offered by the direct vagus ramus (*Di.V.S.C.*) which joins the splenic plexus contribution to the 'anterior coeliac plexus.' For the large nerve (*s.v.c.p.*) which crosses the coeliac ganglion horizontally contributes to the innervation of the left suprarenal gland and kidney. In plate 2, however, there is no doubt as to the presence of direct vagus branches (*Di.V.Ad.*) to the left suprarenal plexus. Three such branches join the sympathetic nerves which make up the capsular plexus of the left suprarenal gland. These vagus branches, though arising from the posterior cord, are fundamentally no different from the suprarenal branches of the anterior cord described by Uchida, since each cord contains both right and left vagus fibers, as has been pointed out in the first part of this paper and as is evident in plate 1. That the capsular nerves, in which the vagus branches in plate 2 are distributed, contribute to the parenchyma of the gland has been shown by Alpert ('31) and others. As to the action of the vagus fibers on the adrenal gland, Goldzieher ('29) considers them as inhibitory to the medulla and secretory to the cortex. The findings of Hoshi ('27), and Kure, Wada, and Okinaka ('31) disagree with Goldzieher's observations, however.

The extensive nerve supply to the suprarenal gland and its intimate relations with the inferior phrenic and renal plexuses are very prominent in plate 2.

The diaphragm. According to Kollmann (1860), Bourguery (1844) described contributions to the phrenic plexus from the posterior vagus cord. Hirt ('24) describes a small gang-

lion in the human foetus which receives contributions from both the posterior and anterior vagus cords, and which gives a branch to the diaphragm. Uchida ('28) describes a branch from the anterior vagus cord to the left inferior phrenic plexus. In plate 1, a nerve (*Di.V.P.*) which arises from that portion of the anterior coeliac plexus which is formed by vagus elements (*V.V.C.P.*) anastomoses with the larger of the two inferior phrenic nerves (*i.p.*) illustrated. In plate 2, several direct vagus branches (*Di.V.P.*) anastomose with the sympathetic components of the left inferior phrenic plexus (*i.p.p.*), which has been dissected in greater detail in this specimen.

Hochstetter ('23) illustrates a 'ganglion phrenicum' which is joined to the superior pole of the left coeliac ganglion, and gives rise to the inferior phrenic plexus. Hirt ('24) describes an 'epiphrenic ganglion' on the right side in a human foetus which contributes fibers to the diaphragm and to the right suprarenal gland. This ganglion is joined to the right coeliac ganglion. These phrenic ganglia are undoubtedly portions of the coeliac ganglion which have been pinched off, as in the case of the renal ganglia to be described; they are not related to the vagus nerves.

The gonads. Kollmann (1860) mentioned the possibility of the distribution of vagus fibers to the sex organs, using the embryological anlage of the latter as a basis for his statement. He makes no contribution to this problem, however. In plate 2, a direct vagus branch (*Di.V.I.S.*) enters the large internal spermatic nerve which arises from the inferior pole of the coeliac ganglion, and follows the internal spermatic vein (*i.s.v.*) caudad. The above, though not conclusive, is suggestive of the vagus innervation of the testicle in this specimen. Further work on this problem, which is in the process of being completed, will help to clarify the situation, I hope.

In view of the fact that our knowledge of the exact origin of the internal spermatic plexus is still unsettled, it might not be out of place to note that in the dissection illustrated

in plate 2, there is a clear connection between the splenic and the internal spermatic plexuses through the splenic plexus contribution to the ventral coeliac plexus (*s.v.c.p.*). It is also interesting to observe that there are direct connections between the renal ganglia (*r.g.*), 'renali-aortic ganglia' and the internal spermatic plexus (*i.s.p.*).

The gall bladder. Haller (1766) described fibers to the gall bladder from the plexus on the right hepatic artery. According to Kollmann (1860), Hildebrandt (1831) described contributions to the gall bladder from the posterior vagus cord through the hepatic plexus. Kollmann describes branches to the gall bladder from the anterior vagus cord. McCrea ('25) also describes anterior vagus cord branches to the gall bladder. In my dissections the branches to the gall bladder were not examined.

The intestines. Haller (1766) wrote of large nerves which passed to the superior mesenteric artery and communicated with the offshoots of the semilunar ganglion. Kollmann (1860) pointed out that various anatomists up to his time disputed the presence of vagus fibers to the superior mesenteric plexus. Kollmann states that in most instances the branches of the posterior vagus cord to the small intestine can be dissected out, as is the case with the fibers to the liver, spleen, left kidney, and left suprarenal gland; but because of the labyrinthian nature of the coeliac plexus caudal to the stomach, the details of the studies of the earlier anatomists had to be considered with caution. McCrea ('25) also describes the superior mesenteric branches of the posterior vagus cord in man, the cat, and the dog.

In plate 1, direct vagus branches (*Di.V.S.M.*) have been dissected into the mesentery of the small intestine from the vagus portion of the anterior coeliac plexus (*V.V.C.P.*). In plate 2 two direct vagus branches (*Di.V.S.M.*) pass to the superior mesenteric plexus.

As regards the large intestine, the vagus fibers are distributed to that portion which is supplied by the superior mesenteric artery. But Kuntz, as has been mentioned with reference to the aortic plexus, offers contradictory data.

The kidneys. There were several accounts of the renal fibers of the vagus before Kollmann's time. According to the latter, the right kidney receives its vagus fibers through the right coeliac ganglion; these fibers, like the vagus fibers to the right suprarenal gland, cannot be dissected. Hirt ('24), however, describes branches from the posterior vagus cord of the cat to both kidneys. He also describes 'renal' ganglia in the renal plexuses of the cat, dog, rabbit, and man. In the cat and in man he describes connections between the aortic plexus and the renal ganglia. Hirt ('30) also describes small ganglia in the renal nerves of the frog. McCrea ('25) describes renal branches of the posterior vagus cord in man, the cat, and the dog.

In plate 1, a direct vagus branch (*Di.V.R.*) joins the left sympathetic renal plexus from the vagus portion of the 'anterior coeliac plexus' (*V.V.C.P.*). But this branch is not as direct as that in plate 2 (*Di.V.R.*) which joins the large sympathetic renal nerve, in which the two renal ganglia (*r.g.*) lie. These ganglia are conventionally called 'renal-aortic'; but 'renal' by Hirt. The larger of these two ganglia is connected with the aortic plexus through the thick sympathetic nerve (*s.a.n.*) which arises from the caudal end of the coeliac ganglion. There are also connections between the renal ganglia and the internal spermatic and suprarenal plexuses. In plate 1, a renal ganglion (*r.g.*) can be seen dorsal to the superior border of the renal vein (*r.v.*).

The liver. The left lobe of the liver is supplied by the branches of the anterior vagus cord which pass through the lesser omentum upon the ventral surface of the caudate lobe, and dorsal to the left lobe. These branches are quite large and easily dissected out. They received sympathetic anastomoses from the right coeliac ganglion in an adult dissection which is not illustrated here.

Haller (1766) described the branches of the posterior vagus cord which follow the hepatic artery. Almost all of the investigators who preceded Kollmann described the hepatic branches of the posterior vagus cord; McCrea ('25) describes

them in man, the cat, and the dog. Hahn ('25), in an illustration borrowed from Rüdinger, shows branches from the 'left vagus nerve,' anterior vagus cord, to the plexus on the hepatic vessels.

The pancreas. Haller (1766), Walter (1783), Meckel (1817), and Valentin (1841) described direct pancreatic branches of the posterior vagus cord before Kollmann's time, according to the latter's review. Kollmann considered the pancreatic branches among those which usually passed through the right coeliac ganglion. McCrea ('25) describes pancreatic branches in man. Kuntz ('29) states that "since both the splanchnic and vagus components involved traverse the coeliac plexus, anatomical separation of the sympathetic and parasympathetic supply to the pancreas is impossible."

My failure to find pancreatic fibers in the two dissections recorded is probably due, to a great degree, to having resected the tail of the pancreas early in the course of the dissection. In an infant specimen not recorded here, however, I did succeed in dissecting two long, thin direct vagus branches, which arose at about the same level as did the gastric branches of the posterior vagus cord. From all appearances these two branches passed into the superior border of the pancreas. I have since not been able to uncover any direct vagus fibers to the pancreas to verify those mentioned, and therefore report the latter with hesitancy, unless they are to be explained on the basis of individual variation.²

The spleen. The splenic branches of the posterior vagus cord were frequently mentioned even before Kollmann's time. Kollmann looked upon them as usual occurrences. McCrea ('25) described them in man, the cat, and the dog. In both plates 1 and 2 (*Di.V.S.*) vagus fibers can be seen passing to the splenic artery; but the resection of this vessel early in the dissection prevents one from drawing any definite conclusions with regard to the splenic fibers.

² In an adult dissection, in progress in this laboratory, direct vagus branches to the pancreas have been dissected out, thus confirming the pancreatic branches mentioned above. This observation will be described in a future publication.

It is of interest to note that the nerves which lie on the splenic artery, near its origin, are not all destined for the spleen and pancreas as is usually believed to be the case. Some of these fibers leave the splenic artery to enter the 'anterior coeliac plexus,' and contribute to the formation of other plexuses, such as the left suprarenal, left renal, and left internal spermatic plexuses. This condition is evident in plate 1 (*s.v.c.p.*) and to a less degree in plate 2 (*s.v.c.p.*).

The stomach and oesophagus. Kollmann (1860) states that after giving off several branches to the oesophagus, the dorsal vagus cord enters the abdomen. Henle (1871) states that the dorsal vagus cord gives several fibers to the oesophagus in the abdomen. In plate 1 a vagus branch passes to the inferior end of the oesophagus; while in plate 2 a vagus branch passes to the region of the cardiac sphincter.

The direct gastric branches (*Di.V.G.*) of the posterior vagus cord are shown in plate 1 in greater detail than in plate 2. Their paucity in figure 2 can be explained on the basis that not much attention was given to these fibers because of the desire to follow the other direct vagus branches of the posterior vagus cord. For detailed and accurate descriptions of the gastric nerve supply, Perman ('16-'17), Brandt ('20), and Hovelacque ('27) are recommended.

A common conception that at least one-half of the posterior vagus cord passes to the stomach was proved to be incorrect by both Kollmann (1860) and McCrea ('25). Not more than one-third of this cord generally passes to the stomach. I have been able to make similar observations in both infant and adult dissections.

SUMMARY

1. A nomenclature which takes account of the interchange between the left and right vagus nerves in the thorax is stressed.

2. The coeliac plexus of the left side is described as consisting of an anterior and a posterior layer.

3. The abdominal branches of the posterior vagus cord are classified on the basis of their origin as: a) right branches; b) anterior branches; c) terminal branches.

4. On the basis of their relation to the coeliac ganglia, these branches are classed as 'direct' and indirect.'

5. The distribution of the 'direct' branches of the posterior vagus cord to the aortic, phrenic, splenic, superior mesenteric, left renal, left internal spermatic, and left suprarenal plexuses is described and illustrated on the basis of dissections.

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PLATES

PLATE 1

EXPLANATION OF FIGURE

The ventral aspect of the left superior quadrant of the abdominal cavity of a male infant: The stomach is rotated to the right on the longitudinal axis of the oesophagus, and the tail of the pancreas and splenic vessels have been resected to expose the coeliac plexuses, and to show their relation to the posterior vagus cord and its branches. (The titles of the vagus elements are capitalized.)

ABBREVIATIONS

<i>ad.</i> , left suprarenal gland	<i>l.c.s.</i> , lesser curvature of stomach
<i>a.v.</i> , left suprarenal vein	<i>l.g.a.</i> , left gastric artery (slightly displaced)
<i>c.g.</i> , left coeliac ganglion (not completely dissected)	<i>L.V.</i> , long medial branches of left vagus nerve to posterior vagus cord
<i>D.V.C.</i> , posterior vagus cord	<i>p.</i> , pancreas (tail resected)
<i>Di.V.G.</i> , direct vagus gastric rami (right branches)	<i>r.g.</i> , renal ganglion
<i>Di.V.P.</i> , direct vagus ramus to inferior phrenic nerve	<i>r.p.</i> , left renal plexus
<i>Di.V.R.</i> , direct vagus ramus to renal plexus	<i>r.v.</i> , left renal vein
<i>Di.V.S.</i> , direct vagus ramus to splenic plexus	<i>R.V.</i> , long medial branches of right vagus nerve to posterior vagus cord
<i>Di.V.S.C.</i> , direct vagus ramus joining the contribution from the splenic plexus to the anterior coeliac plexus	<i>s.</i> , stomach
<i>Di.V.S.M.</i> , direct vagus rami to superior mesenteric plexus	<i>s.a.</i> , splenic artery
<i>e.</i> , oesophagus	<i>s.g.</i> , sympathetic gastric nerve
<i>i.p.</i> , inferior phrenic nerves	<i>s.m.a.</i> , superior mesenteric artery, intestinal branch
<i>i.s.p.</i> , nerves to left internal spermatic plexus	<i>s.v.</i> , splenic vein
<i>i.s.v.</i> , left internal spermatic vein	<i>s.v.c.p.</i> , splenic plexus contribution to anterior coeliac plexus
<i>k.</i> , left kidney	<i>v.c.p.</i> , anterior coeliac plexus
	<i>V.D.V.C.</i> , anterior branches of posterior vagus cord
	<i>V.V.C.P.</i> , vagus elements of anterior coeliac plexus

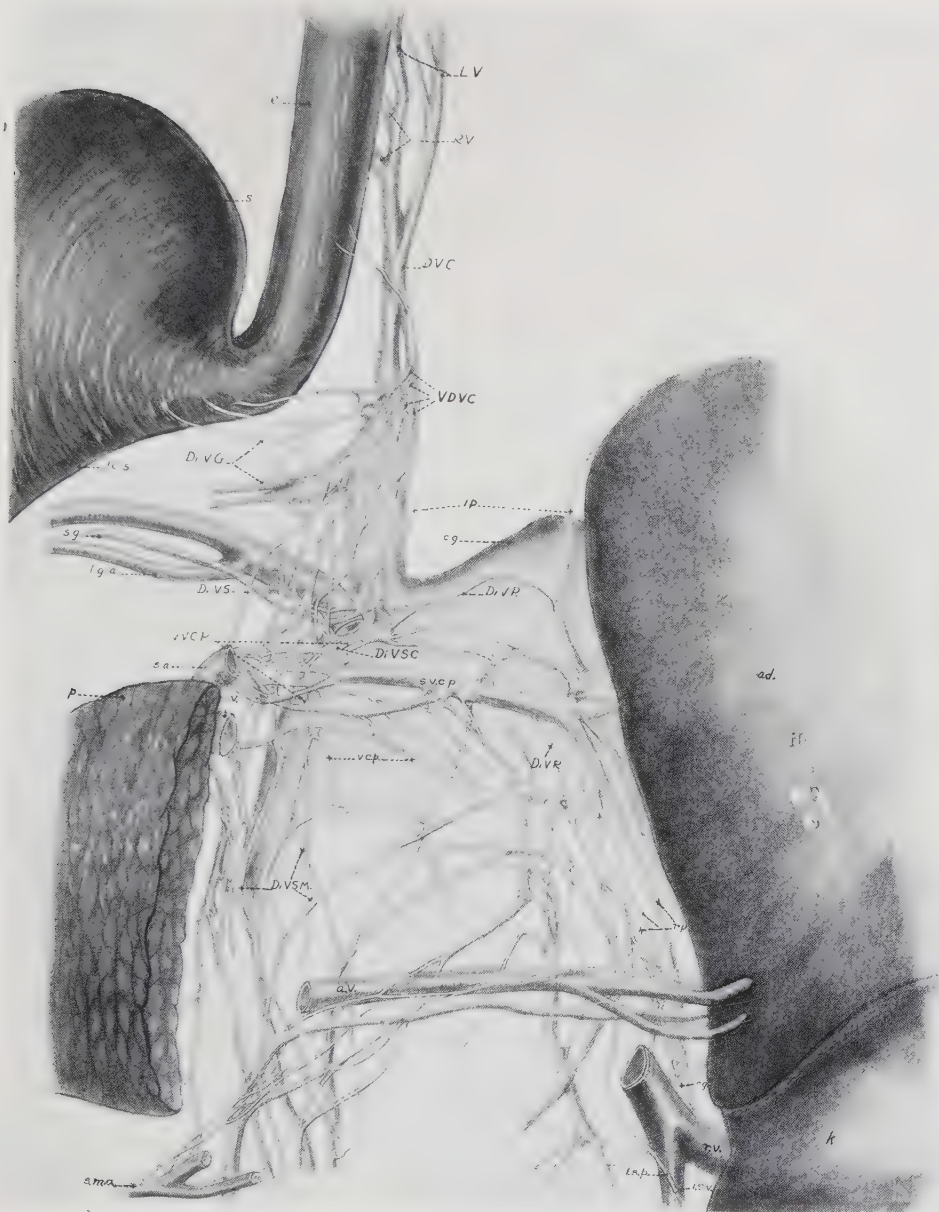


PLATE 2

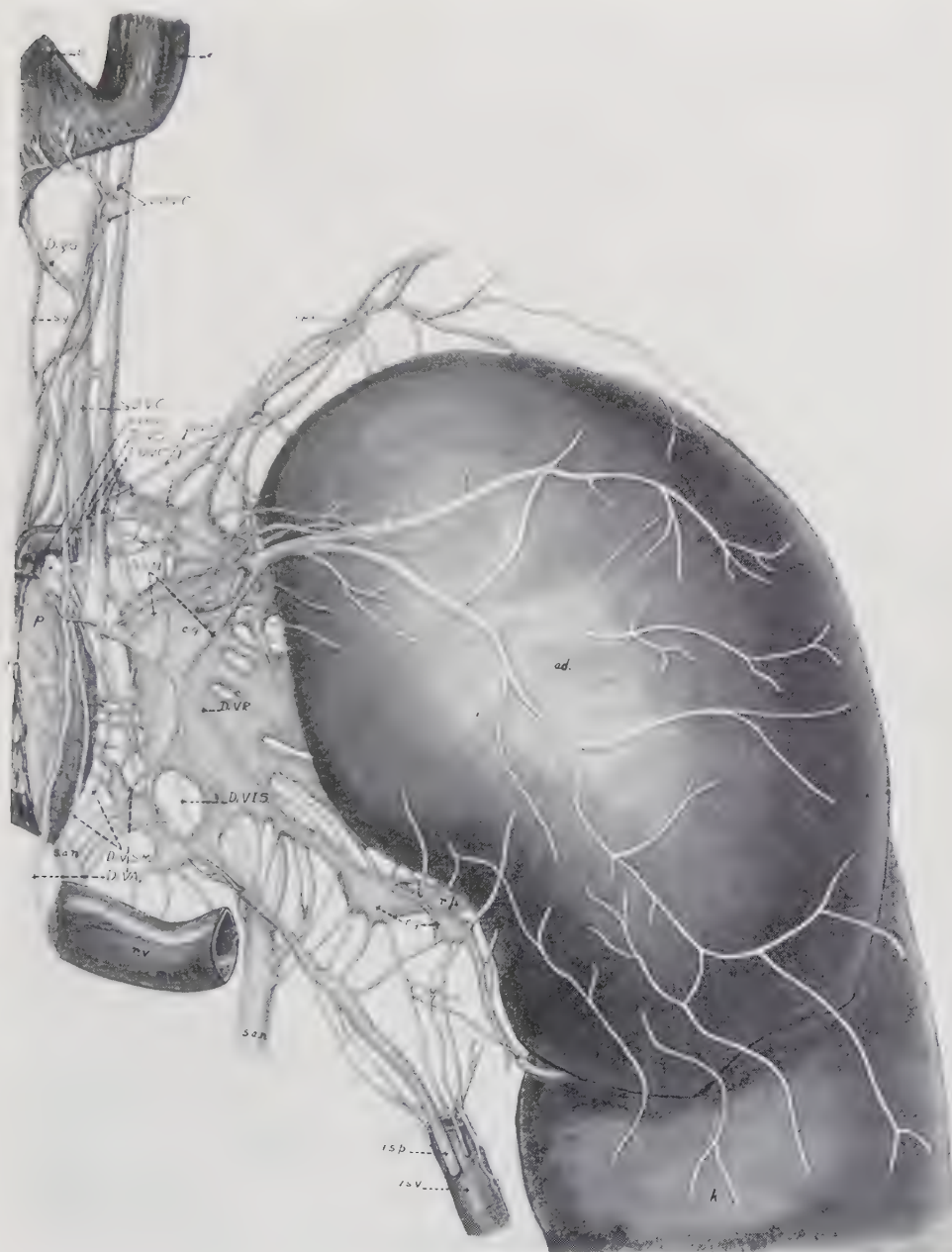
EXPLANATION OF FIGURE

Same as plate 1.

ABBREVIATIONS

<i>ad.</i> , left suprarenal gland and capsular plexus	<i>ip.p.</i> , left inferior phrenic plexus
<i>Di.V.A.</i> , direct vagus rami to aortic plexus	<i>k.</i> , left kidney and capsular nerves
<i>Di.V.Ad.</i> , direct vagus rami to left suprarenal plexus	<i>s.a.n.</i> , sympathetic nerves to aortic plexus
<i>Di.V.I.S.</i> , direct vagus ramus to internal spermatic plexus	<i>T.D.V.C.</i> , terminal branches of posterior vagus cord

(All other abbreviations are the same as in plate 1.)



BOOK REVIEW

NEW TYPES OF OLD AMERICANS AT HARVARD AND AT EASTERN WOMEN'S COLLEGES. BY GORDON TOWNSEND BOWLES. Foreword by EARNEST A. HOOTON. Harvard University Press, Cambridge, Massachusetts, 1932. 144 pp., 20 figures, and 145 tables.

The question of improvement in human physique from generation to generation is of great interest and fundamental importance. Our present knowledge on this subject is based chiefly on European conscript records, Galton's English material, and some Japanese observations, together with mass data for the student population in a few American colleges. It appears fairly well established that there has been a progressive increase, especially in stature, but the explanation of this phenomenon remains somewhat uncertain. While improved environmental conditions are generally held to be chiefly responsible, possible shifts in selection have not hitherto been satisfactorily excluded.

In the present investigation, a more favorable approach was made through actual comparison of parents and offspring. Pearson and Lee had also utilized this method with Galton's data, but their results were somewhat inconclusive on account of the differences in age. The Harvard material used by Bowles was selected from Sargent's excellent series of measurements on 18,000 students at the Hemenway Gymnasium during the period 1880-1917, together with some 2000 earlier records by Brigham. Among these records were found 1461 father-son pairs of data for stature and weight, and a smaller series of 481 pairs for about 30 other measurements. Some further information concerning the grandfathers and great-grandfathers was obtained through questionnaires, but the results are unreliable. Additional records for 315 soldiers of the Revolutionary War from New Hampshire and of 225 Massachusetts seamen prior to 1812 are of interest, but of little comparative value on account of the included shoes and clothing.

The Harvard series, although extending over a long period, presents a fairly homogeneous group, practically all with English, Scotch, Irish, or German ancestry for 2 or 3 generations. Bowles estimates

that probably 80 to 90 per cent are of 'Old American' stock. Parental occupation remained fairly constant, aside from a relative decrease of the manual occupations in the younger generation, with a corresponding increase of the professional classes. At the time of measurement, the mean age of the fathers was 19.65 years; of the sons, 18.51 years. This reflects a progressive tendency to enter college at an earlier age, as has been found also in other American institutions. Since at this age the general population is still growing, the fathers (being older) might be expected to be taller and heavier. But it is a remarkable fact that college groups in general show but little correlation between age and bodily measurements (especially stature), so that, in making comparisons, this age difference can be disregarded.

The mean stature of the Harvard sons (177.5 cm.) is about 3.5 cm. greater than that of the fathers, in agreement with the tendency previously found in various places over the world. When grouped chronologically, the Harvard data show a mean annual increase of 0.08 cm. The greatest increase appears in those born during the two decades, 1856-1875. Apparently the superiority of the sons over their fathers is decreasing, so that (under present conditions) the stature may be expected to become stationary within the next 40 or 50 years.

The increase in stature of the sons is not evenly distributed throughout the body. The total height increases about 2.0 per cent, but the lower limbs increase 2.2 per cent, so the relative sitting height is correspondingly reduced. Moreover, in the segments of the lower limb the thigh shows the greatest relative increase (3.8 per cent). The increase in length of the upper limbs is nearly proportional to stature, but (in contrast with the result in the lower limbs) the arm increases relatively less than the forearm and hand. The breadths and girths of the various parts likewise appear definitely increased in the sons, excepting chest expansion and breadths of head and pelvis. In general, the proportional increases in the various regions correspond roughly to those found in the normal individual growth of the body.

In weight, the Harvard sons average 150 pounds, or about 10 pounds heavier than the fathers. Arranged chronologically, the mean annual increase in weight is 0.21 pound, with the greatest increase in the earlier decades, as was noted for stature. Bowles concludes that, in general, the sons are slenderer than their fathers; but the reverse is indicated by the index of body build (weight in pounds divided by the square of stature in inches). As calculated by the reviewer from the Harvard data, the crude index increased

from about 0.0299 in the fathers to 0.0308 in the sons. This is in close agreement with the change found by MacKinnon and Jackson in 30 years at the University of Minnesota.

The Pearsonian coefficients of correlation (r) between father and son for the various measurements are unexpectedly low. The maximum (for sternal height) is 0.40, while the mean coefficient is only about 0.25. The correlation between stature and weight is somewhat higher for the sons (0.49) than for the fathers (0.44), but the difference is not statistically significant.

Another interesting conclusion from the present study is that, contrary to general belief, when brothers are compared with each other at corresponding ages, the younger brothers average taller and heavier. This difference is accounted for largely (though perhaps not entirely) by the amount of improvement to be expected in the general population during the time between the two brothers. The fraternal differences found in the present study are in general accord with the results of Pearson and Lee, but not with those of Boas (for younger ages).

Turning, now, to the women, the corresponding data were obtained from four different eastern colleges, Wellesley, Vassar, Smith, and Mount Holyoke, providing 570 pairs of mother and daughter. There are some differences in the mean measurements for the various colleges, but they are mostly of questionable significance. The decreasing age of college entrance, as above mentioned for the men, appears likewise in the mean of 18.76 years for the mothers and 17.88 years for the daughters. As was found also for the men, there is but little or no tendency for the various measurements of the college women to show increase when grouped according to age. This indicates that the students represent a selected group, in some respects not comparable with the general population.

In stature, the daughters average 164.53 cm., which is 2.93 cm. above that of the mothers. On the chronological basis, the mean annual increase for all the women is 0.08 cm., the same as was found for the men. The mean weight of the daughters (124.62 pounds) is only about 4 pounds greater than that of the mothers, and the mean annual increase for all the women is only 0.10 pound. Bowles concludes that, since their increase in weight (and also in breadths), does not correspond to the increase in stature, the daughters are slenderer. However, this conclusion is not supported by the above-mentioned index of body build, which remains nearly the same (about 0.0298) in both mothers and daughters. The sitting height index also changes but slightly.

As might be expected from the well-known change in the fashion of clothing, the waist girth of the daughters shows a marked increase (3.56 cm.), with smaller increases in chest and hip girths. The daughters also have greater chest expansion. Unexpectedly, however, the breadths of waist and shoulder appear to increase but slightly, and there is even an apparent decrease of 2.73 cm. in mean hip breadth. Bowles considers this decreased pelvic breadth as perhaps unfavorable to child-bearing. More probably, however, it represents merely a peculiar shift in the softer tissues, due to changes in the pressure of clothing, rather than an actual reduction in the pelvic skeleton. In unaccountable cases of this kind (with data from various schools, extending over long periods of time), possible unsuspected changes in the technique of measurement must also be kept in mind.

The various correlations between the measurements of mothers and daughters, and also the sororal differences, usually appear substantially similar to those found among the men. In absolute measurements, the women are larger than the men in girths of hip and thigh, and closely approach the men also in breadth of hips.

In general, the results of the present study strongly support the thesis that a progressive improvement in physique has taken place in both sexes as a result of more favorable environment rather than through endogenous or hereditary changes. Bowles lists the factors responsible for this improvement in the probable order of their importance as follows: 1) increased medical attention; 2) cultural modernization; 3) better nutrition; 4) more exercise; 5) possible selective mating of parents; 6) occupational changes of parents; 7) climatological or metereological factors.

On the whole, this work is an excellent example of the attractive possibilities in the comparative study of the physical records of students over a long period of time. The employment of statistical methods in such work is especially commendable. The 'probable errors' always serve as a wholesome check against unwarranted conclusions. The few typographical mistakes (and the occasional use of 'data' as a singular noun) are doubtless due to the fact that the author did not have an opportunity to read the proof. The data for Swiss stature in table 36 do not agree with the graph in figure 3. These minor defects, however, are scarcely worthy of mention in reviewing a contribution of such remarkable value in the field of physical anthropology.

C. M. JACKSON.

MATHEMATICALLY PRECISE FEATURES OF EPITHELIAL MOSAICS: OBSERVATIONS ON THE ENDOTHELIUM OF CAPILLARIES

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EIGHT FIGURES

Wax reconstructions of precartilaginous cells of the toad show great diversity of shapes. The facets of contact between cell and cell range from triangles to decagons. When, however, three facets meet at every corner of the cell, which is the usual condition, the number of edges is definitely fixed. If n is the number of facets of the cell, $3(n-2)$ is its number of edges. This formula may be as old as Archimedes. We have discovered it for ourselves, however, by modeling cartilage cells. Its application to cell patterns is extensive, and presumably unfamiliar to histologists. If "sight can teach more in a single day than precept repeated a thousand times," it may be well to visualize this formula in a series of diagrams.

Figure 1, C , is a regular tetrahedron, having four solid angles, all trihedral. It has four faces, and accordingly the formula $3(n-2)$ indicates that it has 6 edges. In cytology it is more serviceable to count each edge twice, since each belongs in common to two adjacent polygons. The formula $6(n-2)$ may then be substituted, to indicate the total number of sides of the facets. In this case, with four triangular facets, $6(n-2) = 12$.

The formula is applicable to shapes such as polygonum seeds, with only three faces and three edges, since these edges meet in trihedral angles (fig. 1, A and B). Two curved surfaces, as in a pea-pod, are insufficient to produce trihedral

angles, and the formula indicates that a two-faced body with trihedral angles does not exist.

Polyhedra with fourteen faces, which is the average number for cells in masses, and with trihedral angles only, have $3(14-2)$ edges, or 36. The total number of sides of their facets is 72, whether as in orthic tetrakaidecahedra there are 8 hexagonal and 6 quadrilateral facets, or whether, as in actual cells, there is a great variety of combinations. For example, let the hidden surface in figure 1, *D*, be a large dodecagon in the plane of the paper. The top surface is also a dodecagon, and the other twelve facets are quadrilaterals. The result is the same—36 edges, or 72 sides of the polygons. The type of diagram in figure 1, *D*, may serve for solids with any number of faces. The basal and top surfaces are then to be polygons each having two sides less than the total number of faces; and the remaining surfaces, equal in number to the sides of the top or basal polygon, are all quadrilaterals. Thus we may visualize a solid with 600 facets, and find that their total number of sides, $6(n-2)$, is 3588. In this and in all other examples—trihedra, cubes, and solids with a vast number of facets making trihedral angles only—the total number of sides of the facets is twelve less than if they were all hexagons.

Fat cells and precartilaginous cells not infrequently present a tetrahedral angle or two, though such angles are unstable and perhaps temporary. Whenever a tetrahedral angle occurs, an edge common to two polygons has been eliminated: in other words, two sides have been lost. For the cytologically rare 5-rayed vertex, two edges or four sides must be deducted. To include all such vertices, let n be the number of polygons covering a solid body, and N be their total number of sides. Their trihedral angles or 3-rayed vertices need no further consideration in this formula, but let a be the number of 4-rayed vertices present, b the number of 5-rayed, c of 6-rayed, etc., the formula then becomes $N = 6(n-2) - 2a - 4b - 6c$, etc.

This formula applies not only to the faceted surface of single cells, in connection with which it was first recognized,

but also to the cellular mosaic covering a *Volvox*, a blastula, or any such object as a cucumber, provided that the surface is entirely covered with cell-polygons, leaving no gap for the stem, or scar at the floral end. It applies also to the epithelial *lining* of any simple completely closed cavity. Since this feature of cells is purely mathematical, it is absolute, admitting of no deviation.

The cell patterns in *Volvox* have been illustrated by Janet with admirable precision.¹ The globe of cells shown in his figure 80 is not quite complete, since there is an octagonal

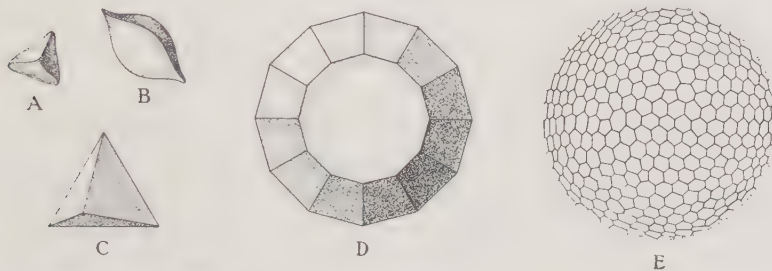


Fig. 1 Solids with facets forming trihedral angles only. For any such solid, the total number of the sides of its facets is 12 less than 6 times the number of facets. *A* and *B*, two views of a 3-faceted polygonum seed. *C*, regular tetrahedron. *D*, tetrakaidecahedron (with a basal dodecagonal facet in the plane of the page). *E*, a 600-faceted spheroid, being Haeckel's rhizopod, *Aulonia hexagonia*.

'phialopore' at the lower pole. If we consider the pore to be occupied by an octagonal cell, this *Volvox* would have 65 facets, and their angles are all trihedral. There are 4 quadrilateral facets, 10 pentagons, 46 hexagons, 4 heptagons, and the one octagonal phialopore, which have altogether 378 sides. With 65 facets, $6(n-2) = 378$.

In the blastula of *Amphioxus* as seen in a commercial model 'nach Prof. Dr. B. Hatschek,' there are 91 cells. At six of the corners, four cells so nearly meet at a point that they may be counted as 4-rayed vertices. All other corners are 3-rayed. When n is 91, and a is 6, $6(n-2) - 2a = 522$. The

¹ Janet, Ch. *Le Volvox*. Troisième mémoire, 1^{re} partie. Mâcon, 1923, pl. IX.

91 polygons in this model are 30 pentagons, 55 hexagons, and 6 heptagons; and their sides total 522. It has been shown in a previous paper that every subdivision of a polygonal cell by a septum so oriented as to make 3-rayed vertices, will add just six sides to the mosaic.² The two daughter cells together will have four more sides than the parent; and two adjoining cells will each receive one added side. Thus every division adds one cell and six sides to the mosaic. Accordingly, we know that cell division in the blastula of *Amphioxus* may proceed until there are hundreds of cells. They will have exactly $6(n-2)$ sides if there are no 4-rayed vertices, and there will be a deduction of two sides for every 4-rayed vertex present. There will be no deviation from the formula when the blastula invaginates to form the gastrula.

Not unlike the late stage of the blastula of *Amphioxus* as commonly modeled, is the skeletal meshwork of the unicellular rhizopod *Aulonia hexagonia* figured by Haeckel.³ In this organism (fig. 1, *E*) Haeckel found the "meshes regular or subregular, hexagonal, often intermingled with a variable number of pentagonal or heptagonal meshes." In his figure it is seen that three sides meet at every vertex, and although the foreshortened marginal polygons become somewhat obscure in the drawing, the figure is consistent with that of a globe having exactly 600 facets. It can then be stated definitely that, although the number of pentagons matched by heptagons is variable, there must be twelve unmatched pentagons, or any combination of pentagons, quadrilaterals, and triangles totaling a deficiency of twelve sides. The average number of sides of the polygons in such a 600-faceted sphere is therefore 5.98. Over the general surface of a cucumber the average number of sides of the cells, if there are no vertices with more than three rays, may be 'absolutely and precisely six' as stated in a previous paper.⁴ But if the

² Lewis, F. T. The correlation between cell division and the shapes and sizes of prismatic cells in the epidermis of *Cucumis*. *Anat. Rec.*, 1928, vol. 38, p. 364.

³ Haeckel, E. *Radiolaria*. Challenger Reports, Zoölogy, 1887, vol. 18, part 2, p. 1634, and pl. 111, fig. 1.

⁴ Lewis, F. T. *Loc. cit.*, p. 347.

rounded ends were completely covered by such cells, and there were a million in all, the average sides per cell would be 5.999988—it could not be precisely 6.

When elevations arise on such a surface, the pattern acquires interesting features. In the diagram, figure 2, *B*, it is assumed that, in an area of hexagons, a single hexagon has been raised to a considerable height above the general level. The six surrounding hexagons have become much elongated, but the others are undisturbed. Let the six stretched hexagons divide transversely, with septa oblique to avoid 4-rayed

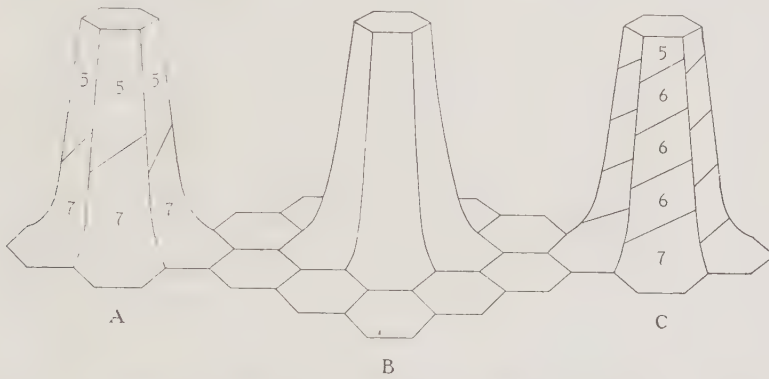


Fig. 2 Diagram of elevations produced in a mosaic of hexagons. Division of the stretched polygons, avoiding 4-rayed intersections, here gives rise to 6 pentagons at the summit, and 6 heptagons at the base, of an elevation. (Horizontal septa at alternating levels would produce 3 quadrilaterals and 3 octagons in place of the 6 pentagons and 6 heptagons, respectively.)

intersections. The condition in figure 2, *A*, is the result. At the top of the column there are six pentagons and at the base there are six heptagons, the average number of sides remaining 6. If further divisions occur in this elevation, always avoiding 4-rayed vertices, then the shaft of the column exhibits cells with six sides (fig. 2, *C*), or with an average of six. In connection with the rounded summit there remains the deficiency of six sides, and in connection with the base, a balancing excess of six sides. The same is true of tubular gland-like depressions, which are merely the inversion of these villous or spiny elevations, as may be seen by turning

the figure upside down. It is true also of elevations, in a general mosaic of hexagons, made by raising a single edge with its connecting corners. In that event, there is no apical cell, and only four cells are stretched, but when they divide there is the deficiency of six sides at the top, and the excess of six at the base.

The loss of six sides at the apex of the column is readily pictured in a variety of forms. If the shaft of a tube is encom-

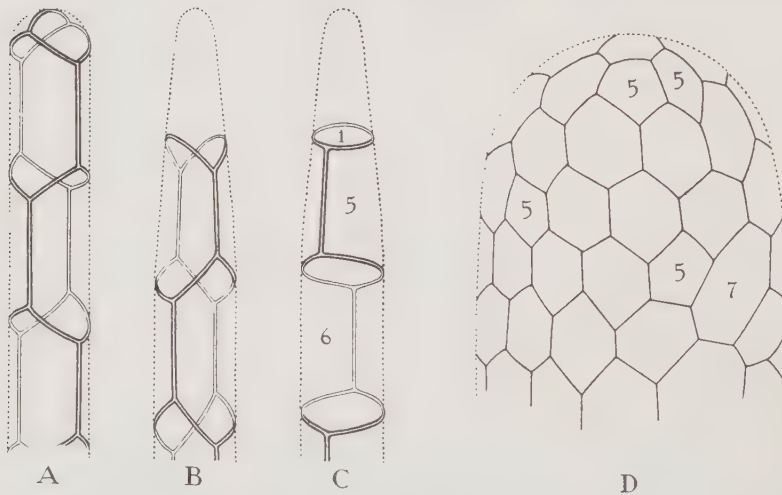


Fig. 3 Diagrams showing the invariable deficiency of 6 sides at the summit of elevations, whether slender and few-celled, or broad and many-celled. (D shows one-half of the summit, with a deficiency of 3 sides.)

passed by two hexagonal cells instead of six, the apex may be formed by two triangular cells (fig. 3, *A*): the deficiency in the number of sides remains just 6. With an apical cell having a two-sided surface toward the lumen (fig. 3, *B*) the luminal surfaces of the two uppermost lateral cells become pentagons. Two pentagons and a two-sided cell, having 12 sides instead of 18, still exhibit the deficiency of 6. Even when the tube is formed by a single file of hexagonal cells wrapped around the lumen (fig. 3, *C*), the apex is made by a cone with but one edge of contact, resting on a pentagon, and

these two terminal cells enter the mosaic with 6 instead of 12 sides. Slender tubes of this sort occur in the endothelial sprouts of blood and lymphatic vessels. When more than six cells surround the tube, as in intestinal glands, the deficiency in sides is not increased. Thus, if a tube is surrounded by 7, 8, or more, hexagons arranged as in figure 2, *C*, the apical cell becomes correspondingly a heptagon, octagon, etc. An octagon and its eight surrounding pentagons have 48 sides instead of 54. But the arrangement of these fewer-sided cells is subject to great variation. More like the fundus of an actual gland is the pattern in figure 3, *D*. This figure represents one-half of a tube, the whole of which is surrounded by 12 cells. With 3-rayed vertices only, the entire tube will have 6 unmatched pentagons, or their equivalent, and by chance three of these are included in the half visible in the figure. There is a fourth pentagon, which is matched by a heptagon, and there might be several of these. With all the variable features, however, the fundus of an intestinal gland, with 3-rayed vertices only, must show a deficiency of 6 sides.

More difficult to visualize is the compensating addition of six sides in the mosaic around the base of every spine and at the orifice of every gland. They do not often form a ring of heptagons as in figure 2, *C*. In fact, one might infer from that figure that if a tubular pit were encircled by seven hexagons instead of six, there would be seven heptagons around the orifice, with an addition of 7 sides. When, however, the orifice of such a pit, surrounded by seven heptagons, is drawn upon a ball of convenient size, or the seven heptagons are marked out around the neck of a globular flask, and the remainder of the spherical surface is covered with polygons forming 3-rayed vertices only, it will be found that these added polygons are *thirteen* sides short of having six sides apiece—not twelve. Hence the net deficiency in sides for the exterior of the sphere is 6; this together with the deficiency of 6 at the fundus of the pit shows that the formula $6(n-2)$ still applies to this hypothetical gastrula-like arrangement.

In the diagram, figure 4, arrows mark the orifices of four pits, each of which has a lumen encircled by 12 hexagonal cells. Twelve hexagonal cells extend out from each pit, spreading over the surface of the page. Clearly, it is quite unnecessary to insert heptagons at the brink. When, however, the space between the pits is covered with polygons forming 3-rayed vertices only, it is seen that a net addition of 6 sides must be

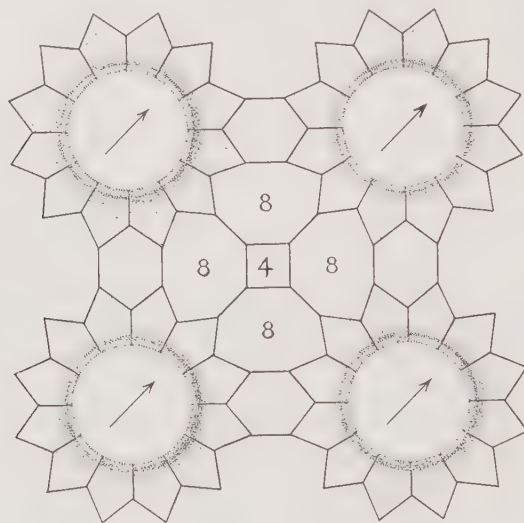


Fig. 4 Diagrams of the orifices of 4 pits, each bounded by 12 hexagonal cells. The lower ends of these hexagons, deflected into the pits, are not seen; their upper ends are spread out flat upon the page. When these upper ends are connected by lines making 3-rayed intersections only, the resulting polygons cannot all be hexagons. There must be an excess of 6 sides for each pit.

made for every pit. Innumerable arrangements are possible. In figure 4 we see four octagons and a quadrilateral—five cells with 36 sides. One such area may be considered as belonging with every pit as suggested by the direction of the arrows. Instead of being pits these orifices may represent bases of villi, or some may be pits and others villi variously intermingled. The result is the same. Further, if the four orifices are not all encircled by 12 cells as in the diagram, but by varying numbers of polygons—7, 8, 6, and 12, for ex-

ample—and the space between them is covered with polygons forming 3-rayed vertices, the excess in sides remains exactly 6 for each pit or elevation.

A tube having three hexagonal cells in its circumference may be inserted into a hexagonal mosaic as in figure 5, *A*, thus avoiding 4-rayed intersections. Three hexagons become octagons, adding 6 sides. Or, as in figure 5, *B*, a tube formed by two hexagonal cells may be inserted, converting two hexagons into nonagons. Again the addition amounts to just 6 sides.

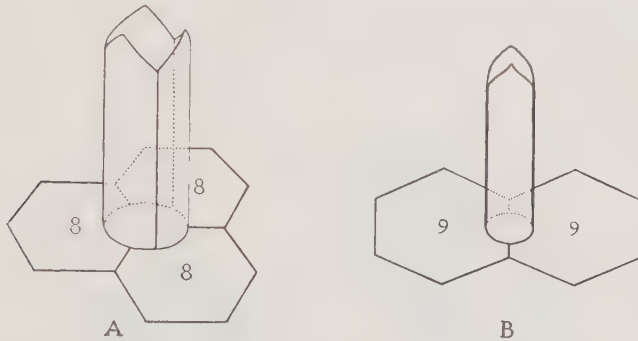


Fig. 5 *A*, a tube having 3 cells in its circumference, inserted into a mosaic of hexagons so as to avoid 4-rayed intersections. *B*, a tube with 2 cells in its circumference similarly inserted. In both cases, 6 sides are added to the cells in the basal mosaic.

From these observations, and many more to the same effect, the inference seems justified that, with 3-rayed intersections only, the production of simple spines, villi, and tubular glands does not modify the formula $6(n-2)$. If a plant—root, stem, and leaves—were completely covered with a mosaic of polygons exhibiting 3-rayed intersections only, there would be exactly 12 unmatched pentagons, or their equivalent, for the entire plant. The same would be true for the intestinal tube with all its villi and glands, provided it were closed at both ends. It remains to consider the effects of orifices, anastomoses, and branching outgrowths.

When two systems of polygons, each having $6(n-2)$ sides, are made to anastomose by a single orifice, in such a way

that none but 3-rayed intersections occur, the resulting system will not lack 24 sides, but only 12. In other words the formula $6(n-2)$, with a new value for n , will be applicable to the combined system, just as it was to each component before fusion. This is true whether the anastomosis be lateral or end to end.

For a simple case of end to end anastomosis, suppose that there are two tubes made of hexagonal cells, with four closed ends each consisting of a terminal hexagon surrounded by six pentagons. Let two of these ends be brought together (fig. 6), to anastomose through the elimination of the terminal hexagons. If the fusion is corner for corner, 4-rayed vertices

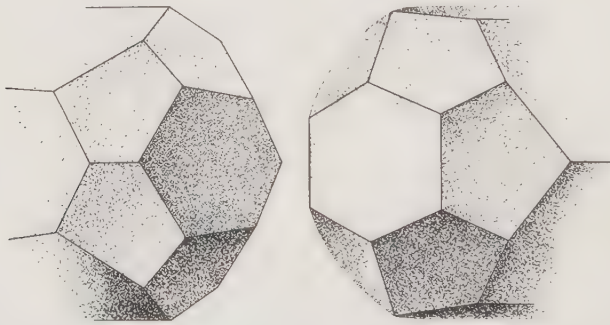


Fig. 6 Two tubes, brought together for end to end anastomosis, oriented to avoid 4-rayed intersections.

arise, to avoid which to the maximum, rotate one of the ends 30° , and then fuse. Two hexagonal cells are eliminated—one from each system—but the number of hexagonal cells is immaterial. Significantly all twelve pentagons become hexagons, and a deficiency of 12 sides is thus eliminated. Hence the combined system, instead of lacking 24 sides, lacks only 12. If the anastomosis were between two of the pentagons, there would be a direct addition of ten sides to the adjacent cells; but with the elimination of two pentagons, each one side short, the whole effect of the anastomosis would be to cancel a deficiency of 12 sides. The result is the same when the anastomosis eliminates many-sided cells. More sides are added to the remaining cells, but the loss of the many-sided,

through which the aperture occurs, reduces the net gain to exactly 12 sides.

The anastomosis need not be through two cells having the same number of sides. In fact, it can be effected through a considerable patch of cells, or through the development of an intercellular space in each system, which space would have the outline of an eliminated cell. In all these cases of a single anastomosis between two closed systems, each having $6(n-2)$ sides, the resulting formation continues to have $6(n-2)$ sides. And this is to be expected, since a body is produced which is covered with polygons having three sides meeting at every vertex:—though it acquires large subdivisions and curious processes, it must conform to the simple formula for all such bodies.

When, however, the two ends of a closed system having $6(n-2)$ sides anastomose with one another, forming a ring; or when one of the ends curves around, and by anastomosing with the side of the system, forms a loop; then, with 3-rayed intersections only, 12 sides are added to the system—its polygons have $6n$ sides. For every additional loop that may be produced, 12 more sides will be added. Thus, if the amniotic cavity should be lined with an epithelium continued over the body of the embryo without interruption, and its cells formed 3-rayed vertices only, its multitudinous cells would be 12 sides short of averaging precisely 6. The stomodaeum and proctodaeum, arising as blind funnel-shaped pockets, would show a deficiency of 6 sides at the fundus of each and a balancing excess of 6 sides at the flaring orifice. The yolk sac, as a closed vesicle lined with polygonal cells making only 3-rayed vertices, would also have a deficiency of 12 sides (fig. 7, *A*). Its fore-gut and hind-gut, as blind pockets, will each present +6 sides around their orifices, and -6 at their blind ends. When, however, two anastomoses are made by the dissolution of the oral and anal membranes, respectively, then, if none but 3-rayed vertices occur, the cells of the entire ecto-entodermal system will average precisely 6 sides.

In the endothelium of a branching system of vessels like that sketched in figure 7, *B*, *open at all extremities*, the expectation is that there will be an excess of 6 sides for every branch, and of 12 for every loop. The fan of vessels shown in the figure, with 43 branches and 8 loops, and none but 3-rayed intersections among its cells, would therefore be lined with cells in number n , having altogether $6n + 354$ sides.

At this point many may agree with Ballowitz that these are "rein theoretische und mathematisch-mechanische Er-

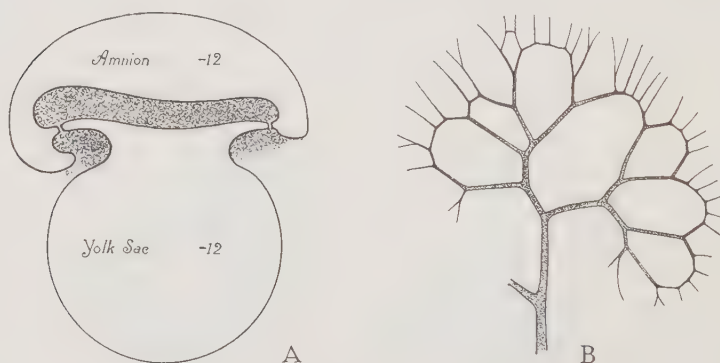


Fig. 7 *A*, diagram of the ectodermal and entodermal cavities. *B*, sketch of some injected vasa brevia of the human superior mesenteric artery (museum preparation by Prof. Thomas Dwight).

örterungen;"⁵ or with Gray that "it is essential to differentiate clearly between biological facts and the expectations based on purely geometrical systems," sharing his surprise that we have found "such a close parallel between fact and geometrical theory."⁶ But in matters such as have here been considered there can be no deviation from 'geometrical theory'—there is no need for an actual specimen. Rashevsky makes the interesting comment: "Whereas in biology, which is yet an experimental science par excellence, the properties of various models are studied by actually experimenting with such models, the physicist frequently satisfies himself with

⁵ Ballowitz. *Anatomischer Bericht*, 1929, vol. 15, p. 418.

⁶ Gray, J. *Experimental Cytology*, 1931, p. 258.

what is called a *Gedankenexperiment*;" and Rashevsky rates the value of such "not only as great as that of actual models, but in certain respects even greater."⁷ Yet to a morphologist there is a sense of security in seeing conclusions exemplified in actual tissues.

The silvered cell-outlines in the walls of capillaries are readily available.⁸ It is astonishing what little use has been made of them, or interest taken in them, as reflected, for example, in Krogh's classic on the capillaries. Accompanying an obscure and murky half-tone are Krogh's statements that "the delimitation of the cells can be made very distinctly visible microscopically by treatment with nitrate of silver and subsequent reduction: the cement will then show up as black lines." The endothelial cells, Krogh says, are "of a polygonal or usually elongated rhomboidal shape compared by Stöhr to steel pens pointed at both ends,"—and that is all.⁹ We would comment that a steel pen pointed at both ends is a hexagon, and that Kölliker's clever comparison with steel pens in 1867 was not Stöhr's only appropriation from his distinguished teacher.¹⁰ The comparison, found in twenty editions of Stöhr's *Histologie* (1887, p. 97, to 1924, p. 153), reappears in Benninghoff's chapter in von Möllendorff's *Handbuch* ('30, p. 26). Except that it implies an arrangement like that in the lower part of our figure 3, *A* and *B*, there is no account in any of the works cited of the arrangement of cells around the entire circumference of the capillary. Not even in Zimmermann's detailed presentation is this feature included.

In pairs of figures side by side Zimmermann has shown the pattern on the upper and under surfaces, respectively, of certain pulmonary capillaries, to indicate that the cells are

⁷ Rashevsky, N. Some theoretical aspects of the biological applications of physics of disperse systems. *Physics*, 1931, vol. 1, p. 144.

⁸ Class specimens, but very good ones, prepared by Professors Wislocki and Weatherford, are the only material used in the following description.

⁹ Krogh, A. The anatomy and physiology of capillaries. 1922, p. 46, and fig. 20, p. 56. Revised ed., 1930, p. 70, and fig. 23, p. 71.

¹⁰ Kölliker, A. *Handbuch der Gewebelehre*. Fünfte Auflage, 1867, p. 596, "die einfach spendelförmigen, schmalen und oft recht langen Zellen, nach Art einer doppelt zugespitzten Stahlfeder tütenförmig eingerollt," u.s.w.

larger on the respiratory side.¹¹ That peculiarity makes it particularly difficult to unite these drawings, clear as they are, in coherent patterns, and Zimmermann has not done so. Neither did the early users of the silver nitrate method, Auerbach and Hoyer, who saw the outlines on both sides of the vessel, by raising and lowering the objective—Auerbach noting that the intercrossing lines confused the picture. He saw, however, that some elongated endothelial cells take a spiral course, which Eberth observed also, finding short stretches of the vessel in which a single cell forms the entire circumference.¹² The silver nitrate patterns are, in fact, 'verwirrend'; but they can be deciphered, especially when drawn on glass tubes. A few of them are as follows.

Figure 8, *A*, is an actual capillary with cells arranged as in the lower part of figure 3, *C*. Its cells are like steel *crow-quill* pens pointed at both ends. Whereas the capillary made of cells like ordinary pens would show two cells in its cross section, alternating with four at their interdigitating ends, this capillary of figure 8, *A*, would show one cell in its cross section, alternating with two at the overlapping ends. If unrolled, each of its cells would be a hexagon. But by no means do we regard this capillary as produced by the rolling up of flat cells! In an early stage the vessels are cytoplasmic tubes with nuclei distributed at rather regular intervals, with no demonstrable cell walls, perhaps with none at all. Then, where the territory influenced by one nucleus meets the territory of another nucleus, a wall is developed, under a tension which avoids 4-rayed intersections. Raindrops on a window-pane, if enclosed by lines making 3-rayed intersections, will yield

¹¹ Zimmermann, K. W. Der feinere Bau der Blutcapillaren. Zeitschr. f. Anat. u. Entw., 1923, Bd. 68, pp. 30-31, Taf. VI.

¹² Auerbach, L. Ueber den Bau der Lymph- und Blut-Capillaren. Centralbl. f. d. med. Wiss., 1865, Jahrg. 3, pp. 178, 179.

Hoyer, H. Ein Beitrag zur Histologie bindegewebiger Gebilde. Arch. f. Anat., Physiol. u. wiss. Med., Jahrg. 1865, p. 244.

Eberth, C. J. Ueber den Bau und die Entwicklung der Blutcapillaren. Würzburger naturwiss. Zeitschr., 1866, Bd. 6, pp. 27-32.

just such a mosaic.¹³ But in the capillary tube (fig. 8, *A*), where the nuclear influence spreading in opposite directions around the tube comes together at the back, there, too, a wall is developed, the cell forming a wall against itself. The same formation is seen in the horizontal vessel in figure 8, *E*, and is undoubtedly common. Yet this longitudinal wall which completes the outline of a rolled-up cell, even in heavily silvered preparations may be absent (fig. 8, *D*, at *x*), so that presumably it forms less readily than the wall between different nuclei. Should it fail to develop in a succession of these cells, they would be like a string of cylindrical beads, with silvered intercellular rings.¹⁴

In a tube constructed of 'rolled' hexagons in the manner of figure 8, *A*, cells may be eliminated through fusion, or added through transverse division, without causing any marked change in the pattern. Fusion would occur by the dissolution of one side of such a loop as extends from *a* to *a*. In place of two hexagonal cells, there would then be one hexagonal cell of double length, spirally twisted about the tube. This conforms to the rule established in the first paper in this series, that in an epithelial mosaic, the removal of any one wall between two polygons of whatever number of sides, results in the loss of one cell and six sides. I know of no evidence that such fusion is taking place in the capillary endothelium, though it remains an interesting possibility. Gräper, as we have previously cited, believed it to be a prevalent method of disposing of 'schwache Schwesterzellen.'

¹³ 'Just such a mosaic' in respect to the average number of sides: other features distinguishing an organic from an inorganic pattern have previously been discussed (Anat. Rec., 1931, vol. 50, pp. 235-265). In a large area of raindrops, polygons have since been drawn around each of a group of 200, and the 200 averaged 5.965 sides. This decimal is variable according to conditions at the periphery (*l.c.*, p. 243). But if instead of falling on a window-pane, the 200 raindrops had encountered a stone, and the net-work had been made to cover its entire surface, then, from the formula already demonstrated, the average number of sides would be fixed at 5.94. No less mechanically do the cell walls develop around the nuclei of an angiocyte.

¹⁴ A succession of three such cells was noted, but after some search it could not be duplicated.

Cell division, on the contrary, certainly took place in producing the capillary tube, and with 3-rayed intersections it

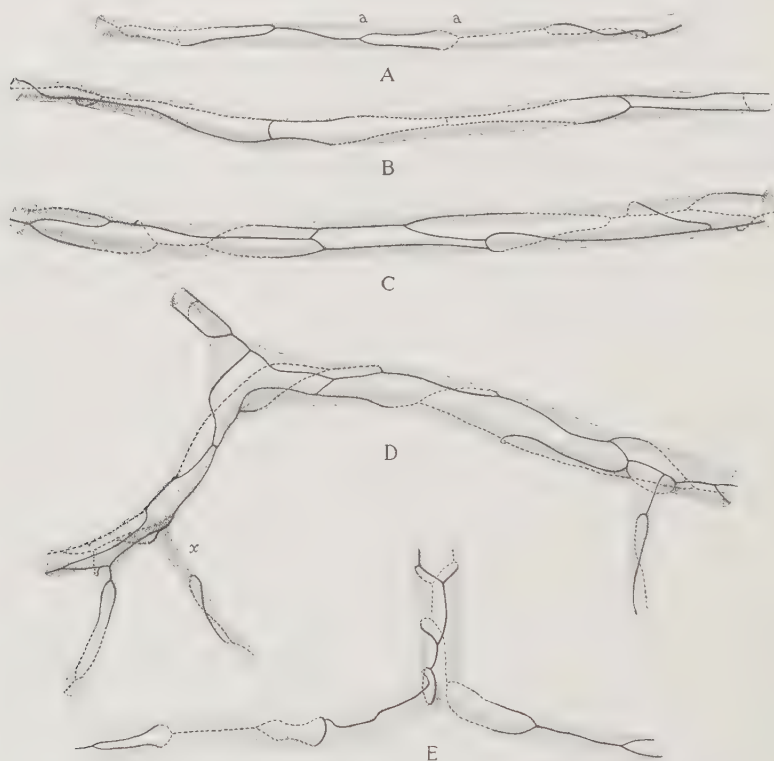


Fig. 8 Silver nitrate preparations of capillaries from the small intestine of a rabbit, to show the shapes of the endothelial cells in the entire circumference. Cell walls on the proximal surface are arbitrarily drawn as continuous lines; those on the distal surface as interrupted lines. $\times$ 425 diameters. *A*, single file of 'rolled' hexagons, with overlapping points appearing as ellipses, the common loop and line pattern. *B*, ladder pattern, with alternate rungs on opposite sides of the tube, being a double row of rectangular hexagons overlapping by half their length. *C*, transition from a two-celled to three-celled tube. *D* and *E*, branching vessels. At *x* a tubular cell undivided by a longitudinal wall.

definitely adds one cell and six sides to the mosaic. The long twisted cell, described in the last paragraph as due to fusion, may well have been the parent of the two cells from which we restored it, and in that case the dividing wall was one side of

the loop from a to a . If with further growth of the vessel, the nucleus of one of these cells should divide and the daughter nuclei move apart, the new wall to be produced between them cannot simply encircle the tube, lest it make a 4-rayed intersection. There must be a twisting of the walls previously laid down, so that the new septum will be half of such a loop as extends from a to a . The twisting seen in the specimen indicates that such readjustments take place. The physical properties consistent with perpetuating and extending this deceptively simple pattern deserve, perhaps, more attention than they have yet received.

Figure 8, *B*, is a capillary composed of elongated hexagonal cells with sides, and not angles, at the ends of their long axes. Such cells would be like steel pens with both ends like the blunt end, so arranged as to overlap those on the opposite side of the tube by half their length. Thus two cells form the circumference of the vessel at whatever level the cross section is made. Transverse division of any cell would cut off a quadrilateral cell, and make the overlapping cell on the opposite side of the tube an octagon. Then we should expect the large octagonal cell to divide, making the quadrilateral a hexagon, and restoring the original pattern. But that procedure would perpetuate the ladder pattern, so characteristic of vegetable tissue but not found in the larger vessels. If, starting from the condition in figure 8, *B*, subsequent division planes were *obliquely* transverse, dividing each rectangular hexagon into a pair of pentagons, the ladder pattern would merge into the steel-pen type. The successive obliquely transverse planes would take a spiral course around the tube, and something of that sort actually occurs.

Figure 8, *C*, shows an ordinary degree of irregularity. Portions of two cells form the cut end at the left, and when completed they have 5 and 7 sides, respectively. Proceeding toward the right, the cells are roughly in pairs around the tube, having successively 7 and 5, 6 and 6, and 8 and 5 sides, ending with *three* cells in the circumference at the right end. These may have 5, 5, and 7 sides, respectively. The transi-

tion from a slender tube, with three or four cells in its circumference, to a great vessel with an indefinitely large number of cells around its lumen is made without departure from the average of six sides per cell. This is easily shown in diagrams. In actual vessels (as in fig. 8, *C*) the taper is so gradual that the change may pass unnoticed, especially since there is alternation between the two-celled and the three-celled circumference before the latter becomes established.

Figure 8, *D* and *E*, show branching vessels, such as can be interpreted only by drawing the cell outlines on a corresponding arrangement of glass tubes. We have learned to expect that every branch will cause an excess of six sides over the general average of six per cell. But although this may be true for an entire system of vessels, it is by no means necessarily exemplified in such small fragments as are here drawn. Proximity to another branch beyond the fragment may cause a deviation; or the part chosen may include a heptagon and miss the balancing pentagon. To avoid these contingencies all cut ends of the fragment must have cells which could articulate with hexagons encircling the tube,—in other words, it must be such as could be set in a frame of hexagons. In that case, were the branches suppressed, the unbranched tube would consist of cells averaging precisely six sides. In such a fragment, exhibiting 3-rayed vertices only, any deviation from the average of 6 is an effect of the branching.

Figure 8, *E*, fulfills the required conditions. A vertical tube, formed of two hexagonal cells of the conventional steel-pen type, divides into two tubes, each of which is a single file of 'rolled' hexagonal cells. We consider such a Y as a bent tube with one branch. The only cells which are not hexagons in this fragment are a pentagon and a cell with 13 sides. These two, with 18 sides instead of 12, show the excess of 6 required by the branching.

Figure 8, *D*, has four branches, three of them one-celled in circumference, and the fourth two-celled, all arising from the main bent vessel which is generally three-celled. For four branches an excess of 24 sides is expected. The articulations

just beyond the cut ends are assumed to be with hexagons. There are ten end cells, partly cut away—3 pentagons, 5 hexagons, a heptagon, and a decagon—with a total of 62 sides. Of entire cells, the fragment includes one with four contacts, two pentagons, a hexagon, a heptagon, and an octagon, three nonagons, and two decagons,—eleven cells with 82 sides. The fragment then consists of 21 cells with 144 sides, being an excess of 18, and not of 24. However, the cell x lacks a septum requisite to complete the net: it is a tubular cell, and not a polygon. If this one missing wall is supplied, so as to make 3-rayed intersections with walls already present, it will add six sides to the mosaic, and the excess of sides becomes 24.

The shapes of some of these cells, especially at the attachment of branches, are very curious indeed. It seems possible to account for them only on the assumption that nuclei are cytoblasts. The nuclei somehow cause the formation of walls bounding their spheres of influence; and under tension, or for other reasons, these walls make 3-rayed vertices. Accordingly, the cell pattern is fixed, to the extent that if it covers completely the surface of any body, or lines uninterruptedly any cavity, it will have $6(n-2)$ sides, when n is the number of cells. In connection with pits and elevations there are loci of few-sided and many-sided cells, but these deviations from the average are strictly limited to a deficiency or excess of six sides in each case. They balance one another, and are included in the general formula.

NOTE.—A beautifully clear picture of the dividing nuclei in a lymphatic vessel, which produced a branch while under observation, has just been published by Speidel (Am. J. Anat., 1933, vol. 52, p. 27). There are no intercellular walls whatever, yet they can readily be supplied around the nuclei (drawn on glass tubes) in patterns like those of our figure 8. This additional fact is brought out. Starting from a tube of hexagons, a conical branch develops as a single cell, with a loop at its base. It has one side instead of six, being an apical cell with a deficiency of *five* sides. Correspondingly the main tube at its base has an excess of only five sides—not six. But the one cell which forms the branch is here both apical and basal: it telescopes the excess and deficiency by canceling a basal side. Let the branch elongate, and its nucleus divide in two, one beyond the other, and the full basal excess and apical deficiency of six will be established.

FUNCTIONAL SITES AND MORPHOLOGICAL DIFFERENTIATION IN THE RENAL TUBULE

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THREE TEXT FIGURES AND THREE PLATES (TWENTY-FIVE FIGURES)

Localization of function in the mammalian renal unit (glomerulus and tubule) and structural modification associated with such function are still poorly understood. This is in large part owing to the fact that the mammalian renal unit is not as accessible to direct experimental attack as that of the frog. This difficulty has led renal investigators to seek in the more accessible units of the kidneys of other vertebrates, chiefly the frog, a more accurate answer to the question of how the kidney performs its function. Varying degrees of validity attach to data obtained from experiments on the frog as applied to the mammal because of the type of kidney as well as the mode of experimentation. It is apparent, therefore, as has previously been clearly shown by the writer, that a study of the structure and function of the vertebrate kidney in general should be undertaken before valid comparative conclusions regarding the mammalian kidney may be drawn. The data presented here are those derived from a further study supplementing that of the writer dealing with the aglomerular and other vertebrate kidneys, the results of which have already been published (Edwards, '28, '29, '30).

MATERIAL AND METHODS

The animals experimented upon with reference to the morphology, histology, and physiology of the renal units of their kidneys were the rat, pigeon, horned toad, turtle, frog, gold-

fish, two kinds of marine fish, earthworm, and the sand-worm, *Nereis*. Into the peritoneal cavity, the dorsal lymph sac (frog) or into the coelome (worms) was injected a recently mixed, 1 per cent solution of ferric ammonium citrate (green scales—Baker or Parke, Davis & Co.) and sodium ferrocyanide. The amount of fluid injected in centimeters and the interval in hours elapsing before fixation, were, respectively, as follows: rat, 2 to 3 cc., 3 to 4; pigeon, 5 cc., 2 to 4; horned toad, 1 to 2 cc., 4 to 5; turtle, 10 cc., 5; frog, 2 cc., 2 to 4; goldfish, 1 cc., 4 to 5; marine fish (the aglomerular kidney of the toadfish, *Opsanus tau*), 5 cc., 5 to 8; earthworm, 0.5 cc., 5 to 6; marine worm, 0.5 cc., 6. Preliminary to excision and fixation of the various kidneys, the rat and pigeon were anesthetized with ether, the frog and turtle were pithed, and the other animals placed for a short time in a 2 per cent solution of urethane.

Series I. For histological study, the kidney of the rat, pigeon, and turtle were rapidly excised and sliced into 5 per cent trichloroacetic acid for 24 hours. For maceration, they were sliced into HCl, 40 per cent for 18 to 20 hours. The kidneys of the frog were excised whole and placed in the respective fixatives. The fish kidneys and the nephridia of the worms were fixed in situ and subsequently dissected free and removed. After fixation, the acid was decanted and 95 per cent alcohol (or distilled water, if macerated in HCl) was added. The alcohol or water, with changes, was allowed to remain 2 or more days, after which the usual histological technique was followed, the sections being cut 8 μ thick and stained with hematoxylin and eosin. The tubules of the macerated kidneys or the nephridia were teased out under water. Some were attached to a substrate and photographed, others were mounted whole on a slide and studied.

Series II. Another series of experiments was performed on the rat, turtle, and frog after it was discovered that trichloroacetic acid precipitated Prussian blue only in the lumen of the distal convolution of the kidney of the various animals studied, except as follows: in the rat's kidney it was precipi-

tated in the cells and lumen of 2 to 3 mm. of the proximal convolution and in the lumen distal to this portion; in the frog it was found in the lumen of this convolution, but not in its cells. In this series, therefore, the ferric ammonium citrate and sodium ferrocyanide were injected separately as well as mixed, and more elaborate methods of fixation were used in order to reveal their presence and distribution in the renal tubule.

1. Rats—average weight, 175 gm. A certain number of these (chiefly males, because the ferric salt is toxic to females) were given intraperitoneal injections of 25 mg. of ferric ammonium citrate (larger doses are toxic even for the male rat) and treated as follows: at the end of 3-, 6-, 12-, 24-, and 48-hour periods: the rat was anesthetized with ether, after which the kidney and aorta were exposed. The latter was clamped just above the renal arteries and a hypodermic needle inserted into it below the clamp. Then, following in principle the method used by Holton and Bensley ('31), 5 cc. of Ringer's solution were slowly injected followed at once by the injection of 10 cc. of a mixture of neutral formalin and absolute alcohol (1 part formalin and 9 parts alcohol). The kidney was excised and sliced into absolute alcohol, where it remained for 24 hours. An equally effective and somewhat easier method which was also used is that of ligating the aorta, sectioning a renal artery, and injecting the fixing fluid through a renal vein. Trichloroacetic acid or HCl was substituted in some experiments for the formalin alcohol mixture. Kidneys injected with HCl were allowed to remain for 20 hours, after which they were washed and kept in distilled water until the tubules were teased out and mounted on a slide under a cover slip, using a technique developed for the purpose. The rats into which the non-toxic sodium ferrocyanide (25 to 50 mg.) was injected at the end of 1-, 3-, and 24-hour periods, were treated in the manner just described.

2. Turtles—average weight, 250 gm. These were given intraperitoneally 100 mg of ferric ammonium citrate and subsequently treated as were the rats.

3. Frogs—average weight, *Rana pipiens*, 25 gm., *R. catesbiana*, 150. These were injected via the dorsal lymph sac with 20 to 30 mg. of either ferric ammonium citrate or sodium ferrocyanide. At intervals of 2, 3, 4, 24, 48, 65, and 100 hours, some of them were pithed, others were anesthetized with urethane and treated as were the rats. In addition, in some experiments, the renal portal vein and aorta were ligated, cut, and the fixing fluid injected through the vena cava. The best results, however, were obtained, especially for demonstrating the intracellular content of ferrocyanide, by injecting through the ureter a solution of 5 per cent trichloroacetic acid containing 1 gm. of ferric ammonium citrate per 100 cc. of acid. If done with care, this method of fixing the renal epithelium does not flatten it sufficiently to prevent reliable microscopic study of the histological preparations which were made of such kidneys.

Certain experiments were performed on the frog in which 50 to 75 mg. of urethane were injected along with the iron salts, or shortly thereafter. At the end of 2 to 3 hours, the kidneys were fixed by each of the methods mentioned above.

All of the histological preparations which were made of the kidney of the rats, turtles, or frogs were placed for a minute or two, after the paraffin had been dissolved from the sections, in a 1 per cent solution of the complementary salt. Slides made of the kidneys of animals injected with a mixture of the iron salts were treated as follows: of three slides made from the same kidney, one was placed in the solution of ferric ammonium citrate, the second in that of sodium ferrocyanide, and the third left untreated. Sections were stained with Mayer's acid carmine or left unstained.

Measurements of the proximal convolution of the rat were made after this convolution had been uncoiled and attached uniformly to a substrate which covered the bottom of the dish in which it had been dissected. The dish was placed under a binocular microscope, one eye piece of which contained a pointer. A ruler was laid across the dish above the tubule and parallel to it, so that the edge of the ruler was

seen by focusing the microscope. By moving the dish, the tubule was placed as desired with reference to the pointer, and by focusing the microscope either the ruler or the tubule was brought into focus with the pointer and readings taken.

RESULTS

General distribution of Prussian blue

Series I. Functional sites as revealed by the presence of ferric ferrocyanide (Prussian blue) in the epithelium of the renal tubule or in its lumen following injection of a mixture of equal parts of a 1 per cent solution of ferric ammonium citrate and sodium ferrocyanide and fixation of the kidney in a 5 per cent solution of trichloroacetic acid.

1. The glomerular capsule and neck. No Prussian blue has been observed here in the kidney of any animal studied, despite the fact that the histological preparations which were made of the various kidneys were treated with a 1 per cent solution of ferric ammonium citrate or sodium ferrocyanide. In the case of the ferric salt (ferric ammonium citrate injected), the absence of Prussian blue may be taken as definite proof of the absence of the ferric ion in this area, since a precipitate of Prussian blue is invariably formed when a ferrocyanide solution is added to a solution of a ferric salt, no matter how dilute the latter may be. However, no definite conclusion regarding the presence or absence of ferrocyanides may be made, since solutions of ferric salts added to dilute solutions of ferrocyanides may result in the formation of alpha, beta, or gamma soluble Prussian blue.

2. The proximal convolution. Prussian blue is not visible in the cells of this convolution in the kidney of any animal studied, except in that of the rat and horned toad. In that of the former, its presence is sharply localized in particulate form in the cells and lumen of 2 to 3 mm. of this convolution. The segment constituting the 2 to 3 mm. is found in the first part of its distal third (fig. 1, part whose entire diameter is stippled). In the portion 6 to 8 mm. long extending from the

glomerulus to the segment containing Prussian blue, no trace of the latter has been seen with certainty either in the cells or lumen. It is abundantly present, however, in the lumen with but little present in the cells, in the part continuous with the segment containing Prussian blue and terminating at the

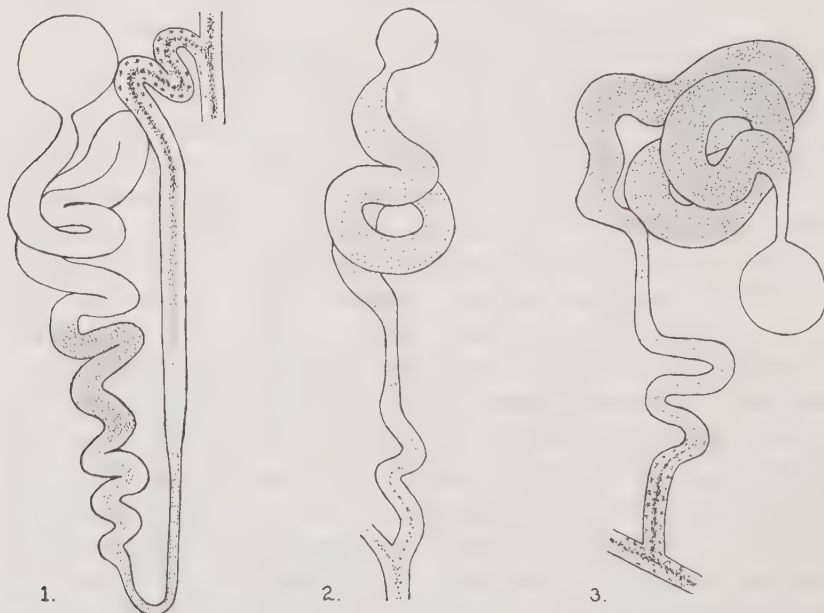


Fig.1 Diagram of a renal unit of the rat's kidney, showing the distribution and relative size of Prussian blue particles as they appear in the tubule when the kidney is fixed during the active excretion of iron salts.

Fig.2 Diagram of a similar unit in the turtle's kidney, showing by stipple the relative content of iron particles as seen in various parts of the tubule.

Fig.3 Diagram of a renal unit of the frog's kidney, showing the distribution of Prussian blue. The presence of iron in certain cells of the distal convoluted is shown by patches of stipple in its margin.

beginning of the thin segment. In the lumen of the proximal convoluted in the kidney of the other animals studied, only that of the frog's kidney contains appreciable amounts. This may mean that some iron is normally excreted through the glomerulus, as indeed has been found by Dr. A. N. Richards (personal communication) by testing fluid taken from the glomerular capsule.

3. The thin and intermediate segments. The thin segment is a development found chiefly in the mammal (Huber). In the rat's kidney it is conspicuously and uniformly present (fig. 27, lower left corner forming the left side of the somewhat crescentic *V*). Prussian blue particles of the same size and appearance as those seen in the described and measured segment of the proximal convolution, occur in the lumen of this segment. Similar particles are also seen in the lumen of the ascending limb, as is indicated in figure 1, with which, for a picture of its actual morphology, compare figure 27, lower left corner on the right side of the crescentic *V* and extending to the indentation just above its intersection with the lower part of the proximal convolution. Since this limb is not designated as such or in equivalent terms in the lower vertebrates, it will not be mentioned under a separate title. It appears to be functionally present in the lower vertebrates.

The intermediate segment which is regularly present in the kidney of the reptiles, frogs, and fresh water fish examined, but only occasionally in that of marine fish, contains particles of Prussian blue in its lumen, only when the lumen of the proximal convolution contains them. In this series of experiments they have been observed in the kidney of the animals mentioned only in the lumen of the frog's kidney. This is believed to be largely the result of inadequate fixation of the kidney with reference to the visible retention of iron, as will later be indicated.

4. The distal convolution. This convolution is present and of considerable length in the kidneys of representative animals in the chief classes of vertebrates except in that of the marine fish. In the latter, it is represented by a short segment, 12 to 16 cells long, continuous distally with a duct. Prussian blue is found in concentrated form in the lumen of this portion and in that of the adjacent duct in the glomerular kidney of the marine fish, *Fundulus*. The excretion of iron by the aglomerular kidney of the toadfish is so slight as to make impossible definite statements concerning its concentration in the lumen of the tubule. It is poorly excreted by

both the glomerular and aglomerular kidneys of the two fish mentioned, as well as by other aglomerular fish kidneys (Verne, '22), although the glomerular kidney excretes relatively greater amounts than the aglomerular kidney. From this difference in the excretion of iron by the two types of kidneys the conclusion need not be drawn that the glomerulus is the site of iron elimination, since the presence of the latter may influence the renal epithelium in a way favorable to the excretion of iron without itself being involved in its elimination.

Prussian blue is found precipitated in aggregates of variable size in the lumen of the distal convolution in the kidney of the rat, pigeon, horned toad, turtle, frog, goldfish, earthworm, and marine worm (figs. 14 to 24). In all instances, the larger size of these aggregates as compared with those observed in either the cells or lumen of the proximal convolution indicates that the reabsorption of water has occurred permitting this marked aggregation and relative congestion the results of which are clearly shown in figures 20, 22, 23, and 24, where certain lumina (central in the figures) contain Prussian blue photographed as black.

A peculiar circumstance was afforded by the presence of iron in relatively large amounts in the lumen of the distal convolution at a time when little or none was observable in either cells or lumen of the proximal convolution, and also when it was being actively excreted in the urine at the time of fixation of the kidney. If one salt or a part of it were eliminated by the glomerulus and the other by the cells of the proximal convolution, or both by either route, iron should be present at least in the lumen of the latter. The possible significance of this peculiarity will be mentioned in connection with the results obtained by using more particular methods of fixation.

Series II. Functional sites as revealed by injecting the fixing fluid intravascularly into the kidney or through the ureter when ferric ammonium citrate, sodium ferrocyanide, or a mixture of equal parts of these two salts was being ex-

creted. When the ferric salt is injected into the rat, essentially the same picture is seen in histological preparations of its kidney, following its treatment with sodium ferrocyanide, as that obtained and already described as a result of an injection of a mixture of the two salts without counter treatment; namely, 2 to 3 mm. of the distal third of the proximal convolution contain Prussian blue in the cells and lumen. In this connection, it may be well to emphasize the fact that the iron salts were injected intraperitoneally into normal animals rather than intravascularly into those animals subjected to unusual conditions such as very low blood pressure or extreme dehydration. Even so, the results recorded here from experiments on the rat are in essential agreement with those obtained by Holton and Bensley ('31) from experiments on the rabbit. In the remainder of the tubule distal to these 2 mm., Prussian blue occurs only in the lumen, except for certain cells of the distal convolution, which contain it, as will later be described. A diffuse, pale blue color can, in some preparations, be seen throughout the length of the proximal convolution, especially between the blue granules present in the cells and lumen of the described 2 to 3 mm. A reasonable conclusion is that the diffuse blue is owing to the formation of soluble Prussian blue by the action of acid on the ferrocyanide or on the more dilute iron solution present in the lumen of the tubule at the time of fixation.

In the kidneys of the turtle and frog, the entire proximal convolution contains particulate Prussian blue uniformly distributed in the cells throughout its length, but in very different amounts. The cells of the proximal convolution of the frog contain twice as much or more than those of the proximal convolution of the turtle when sections of their kidneys as well as whole tubules are compared. This observation supports that made by Cordier ('28), namely, that iron is excreted very poorly by the kidney of the turtle. Quantitative determinations of the total percentage excretion of ferric iron by the two animals is as follows: turtle, 1 to 3; frog, 60 to 70. The rat excretes 20 to 30 per cent. It is significant that the

content of blue granules in the cells of the proximal convolution in the two types of kidney as estimated by a microscopic study, should constitute an indication of the relative amounts actually excreted (figs. 1 to 3, relative contents shown by stipple in the area indicated by the outlined tubules). Also, when the length of the proximal convolution in the kidney of the rat, turtle, and frog is compared and the portions containing Prussian blue noted, it is found that in the rat's kidney, 2 to 3 mm. of its total length contain blue granules; in the turtle's kidney, 1.5 mm., and in that of the frogs, 3.8 mm. In the latter two, these figures represent the entire length of their respective convolutions. If it be assumed that all of the renally eliminated iron is excreted by the proximal convolution as described, the efficiency of this convolution in the kidneys of the three animals (in terms of the number of millimeters involved when allowance is made for the tremendously larger number of tubules in the kidney of the rat and turtle) is very much greater in the frog's kidney and least in that of the turtle, which is representative of the highest type. If, however, the glomerulus constitutes an auxiliary site for this elimination, as is indicated by Richards' experiments of the frog's kidney, then the difference in efficiency between the proximal convolution of the rat and frog becomes less significant.

THE REABSORPTION OF PRUSSIAN BLUE

That the distal convolution not only constitutes the site for the resorption of water, especially in its proximal half, but also of iron in its distal half and duct portion, is indicated by the fact that certain cells of this convolution contain blue particles identical in size and appearance with those present in its lumen as described. Such particles are diagrammatically shown in figures 1 and 3 by the stippled patches in the white margin of this convolution which represents its cell wall. Photographic representation of the actual presence of iron in the cells of the distal convolution and duct portion with which the former is continuous, is shown, in figure 28,

in the cells of the transection to the right of *Z* and in those of the duct to the right of this transection. Since the appearance of the blue particles in the cells is identical with their appearance in the lumen, their presence in the cells is believed to be the result of their resorption from the lumen (compare, in fig. 21, the appearance of Prussian blue in the lumen of the transection to the left of *Z* with that in the cells of the transection in fig. 28, at the right of *Z*).

THE DETECTION OF FERROCYANIDE IN THE TUBULE

The presence of this complex ion in tissues in general and in renal epithelium in particular is difficult to detect, except by the use of special methods. Certain aspects of this difficulty are worthy of note. Its presence is easier to detect by ordinary methods of fixation following the injection of a mixture of the two salts than after that of sodium ferrocyanide alone. An indication of this is found in the rat's kidney after the injection of the latter. Despite careful methods of fixation, involving counter treatment with a ferric salt, no portion of the epithelium of the tubule contains with certainty appreciable amounts of Prussian blue. This is probably the result of the formation of the soluble forms of the latter, referred to by Hackh ('29) as alpha, beta, and gamma soluble Prussian blue, which are obtainable in test tube reactions when a ferric salt is added to dilute solutions of a ferrocyanide. The fact that when a mixture of the two salts is injected into the rat, one segment only of the proximal convolution is laden with blue granules (showing the presence of both ions) when the kidney is merely excised and placed in an acid-containing fixative, is contingent upon a definite specificity for the ferric ion by this segment and the fact that when ferric solutions are treated with ferrocyanide, a precipitate of Prussian blue is invariably obtained. This reaction shows that ferrocyanide as well as the ferric ion, when both are present in the blood, are excreted through the designated segment, although no observations made thus far on the excretion of ferrocyanide alone reveal a definite locus for its excretion as is shown for the ferric ion.

There is no segmental specificity for the excretion of iron salts in the proximal convolution of the kidney of either the turtle or frog that may be regarded as comparable with that of the 2 to 3 mm. of this convolution in the rat's kidney as described and depicted. In the frog's kidney, ferrocyanide is found distributed intracellularly in granular or globular form throughout the length of its proximal convolution when the fixing fluid is injected through the ureter, but to a very much less extent when such fluid is injected intravascularly. It is clear that the method of fixation is very important in attempting to ascertain the distribution of the ferrocyanide. Associated with this necessity for a special mode of fixation is the functional significance of the conditions requiring it. These conditions are being investigated.

When ferrocyanide is present in the lumen of the tubule, as indicated by its subsequent precipitation as Prussian blue by a fixing fluid to which a ferric salt has been added, it is relatively unconcentrated in the lumen of the proximal convolution in comparison with the distal convolution. In the latter it is highly concentrated. The presence of ferrocyanide in the glomerular capsule has not been demonstrated. This fact cannot be regarded as conclusive evidence that it is not eliminated by the glomerulus.

THE EFFECT OF URETHANE

The primary effect as observed histologically in the tubule of the kidney of the frog is that of increasing two-fold or more the content of Prussian blue in the cells and lumen of the proximal convolution. The lumen of the distal convolution also appears to contain more of it in concentrated form. To what extent, if any, urethane increases the resorptive power of the cells of this convolution and promotes the elimination of iron or other solids in solution by that of the proximal convolution is now being studied.

THE EFFECT OF INJURY

Among the rats from a stock supply in which no signs of renal injury had been detected previously, one was found in which the renal tubules showed a marked regional necrosis. This rat had been given 48 hours before fixation of its kidneys, 25 mg. each of ferric ammonium citrate and sodium ferrocyanide. The presence within the rat of the injected salts could not have been responsible for the injury, since in the kidneys of normal rats subjected to similar treatment no comparable necrosis was found (compare fig. 25, normal cortex, with fig. 28, necrotic cortex). The urine excreted during this period amounted to 20 cc. (more than twice the normal volume) and contained appreciable amounts of albumen.

Both kidneys of this rat were examined as follows: one-half of each kidney was prepared for histologic study and the remaining halves were macerated. The isolation of the macerated halves revealed that approximately 90 per cent of them were necrotic. The necrotic portion extended from the glomerular capsule for about two-thirds the length of the proximal convolution (fig. 27, bow-shaped portion at the right). The relatively uninjured portion extends, as shown in figure 27, from the indentation above the numeral and slightly to the right of it, across the picture forming an 'M.' The thin segment is shown continuous with the left side of the 'M' and terminates abruptly at the bend (lower left corner). The ascending limb and distal convolution constitute the remainder of the tubule. No significant signs of injury were found in other parts of the kidney. The condition of the renal epithelium of the injured portion of the proximal convolution is best shown in the right third of figure 28 and the evidence of repair, by mitotic figures, one of which, in anaphase, is seen at X (others at X'). Such figures are not found in the epithelium of the relatively uninjured portions of the tubule, sections of which, exclusive of X' are shown in figure 28, in its left third and also below the glomerulus and to its right.

Prussian blue was found occupying the same locus in the proximal convolution of this injured kidney as described for

the normal. A photographic representation of its characteristic appearance in the form of fine particles in the cells of this locus as well as in the cells of the proximal convolution in the kidneys of other vertebrates, is afforded by the dark, stippled appearance of the cells in the three adjacent tangential sections, the largest of which is shown in figure 28, above *Y*. In contrast with the appearance of Prussian blue in the cells of the proximal convolution is its appearance in some of the cells of the distal convolution and duct portion. A transverse and longitudinal section of the latter, respectively, are shown in figure 28, in the section at the right of *Z* and in the duct next to it. In the cells of the right wall of the transection and the left wall of the duct are seen black granules (Prussian blue) typical of their appearance in some of the cells of this portion of the tubule in the normal kidney of the rat and frog following injections of iron salts.

Active excretion of iron by this injured kidney at the time of fixation had stopped, as judged by its absence from the lumen of the distal convolution and duct portion. The urine was collected for the whole 48-hour period before testing it for the presence of iron; therefore, in this instance, it is not known when the excretion of iron was terminated. The urine of normal animals, however, does not contain significant amounts of iron after 20 hours. The urine from the injured kidney contained considerable ferrocyanide with but very little of the ferric iron.

In the lumen of the thin segment, ascending limb, and distal convolution of some tubules is present a precipitate identical in appearance to that seen in the lumen of the injured portion of the proximal convolution (fig. 28, upper right hand corner). In contrast to the precipitate in the lumen of the injured portion, this precipitate contained fine blue particles indistinguishable from those observed in the cells of the described portion of the proximal convolution. Their appearance in this form is indicative that, owing to the presence of the precipitate in which they were embedded, they were prevented from forming aggregates by dehydration. Since the

precipitate in the lumen of the injured portion of the proximal convolution which was proximal to the segment designated as iron-excreting contained no particles of Prussian blue, it appears that the particles observed in the precipitate in the lumina of those parts of the tubules distal to the proximal convolution had their origin solely from the iron-excreting portion of the proximal convolution. There is not available from studies of normal rats the slightest evidence that iron is excreted by the glomerulus or that it is resorbed from the lumen of the proximal convolution. This is confirmed by the observations made above of the tubules of this injured kidney. The apparently normal condition of this renally injured rat, despite the albuminuria found, is probably the result in considerable part of the presence of normal glomeruli and tubules which were necrotic only in part.

MORPHOLOGICAL DIFFERENTIATION

Actual photographs of various renal units (figs. 4, 5, proximal convolutions only, 6 to 13, and 28) are shown because of the probable physiological significance associable with their morphological differentiation. In the nephridia of the two worms studied (figs. 16 to 19) there is a certain morphological and physiological similarity to the vertebrate tubule, especially as regards the proximal and distal segments and the concentration of iron in the lumen of the latter. It is also noteworthy that structurally, when compared with the renal units of other vertebrate kidneys, the less differentiated tubules, both morphologically and cytologically, are those present in the functional pronephros of *Fundulus* (Armstrong, '32) and similarly in the mesonephros of marine fish (Edwards, '28, '29) and in that of the chick (Boyden, '27, '31). According to the theory of glomerular function, the aglomerular tubule should be the most differentiated, since it must perform the entire function of renal excretion. If it should be argued that environmental factors of auxiliary sites for the elimination of urinary constituents form the basis for the explanation of the relative undifferentiation of these

tubules, still the nephrostome or glomerulus is present in connection with the more differentiated tubules, however slight this relative differentiation is in the renal units of some animals.

Armstrong has shown that the onset of function of the pronephric tubule in *Fundulus* is coincident with the development of venous capillaries along its surface and that the glomerulus does not develop until after function is established in the tubules. The writer (l.c.) has discussed the significance of the venous blood supply to aglomerular kidneys and also directed attention to the probable significance of the altered blood which supplies the tubules through the efferent artery in adult metanephroi. It is singular that in the kidney of the young goosfish where glomerular tubules are present these degenerate while new, aglomerular tubules arise entirely independent of those degenerating and finally make up the entire content of functional tubules in the adult mesonephros.

Wherever the renal tubule is modified at its proximal extremity by a nephrostome or glomerulus, a very pronounced differentiation, with certain exceptions already noted, is manifest in the tubule either morphologically or cytologically or both as compared with aglomerular tubules. One of these morphological and cytological aspects of differentiation is seen in the thin and intermediate segments present in vertebrate kidneys exclusive of marine fish. While it is not known what function is performed by these segments, that of non-specific, physical readjustment between blood and urine ascribed by Bensley and his co-workers to these segments may be proved to be correct when more facts concerning the actual significance of their presence shall have been established. It has been conjectured that the thin segment of the mammalian renal tubule may be responsible for the hypertonicity of mammalian urine as compared with the hypotonicity of the urine of lower vertebrates. But, as far as facts are available, any one of several structural or metabolic factors might equally well constitute the underlying cause. Conceivably such a cause might be the greater length of the proximal convolution

in the mammalian kidney and its functional differentiation as manifest segmentally unaccompanied by obvious cytological change. It is probable that the thin and intermediate segments have comparable function in their respective tubules.

Analysis of the data available shows they support Suzuki's ('12) division of the renal unit into the filter unit (glomerulus and capsule), the unit of secretion (proximal convolution) and the unit of resorption (distal convolution). It has not been reliably shown as yet that the cells of the proximal convolution are capable of resorption of either water or solids, with the exception of glucose. Unequivocal proof of the locus of the resorption of the latter is about to be offered by Richards and his co-workers (personal communication). Nishi's ('10) experiments on the mammalian kidney in which he separated cortex from medulla and found appreciable amounts of glucose in the cortex, but little or none in the medulla, reveal nothing concerning what part of the tubule may have been active in its removal. This is said in the light of a knowledge of the arrangement of the tubules with reference to the medulla in the mammalian kidney. Any valid separation of cortex from medulla would include in the latter the thin segments and parts of ascending or a few descending limbs of the medullary loop as distinct from the ducts. The ducts would constitute the bulk of the medulla examined.

It is also held that in the kidney of marine fish the tubules of which do not contain a distal convolution comparable with that in the kidney of either fresh-water fish or the higher vertebrates, resorption occurs in the cytological equivalent of the proximal convolution. But in the kidney of *Fundulus*, a marine fish, following an injection of iron salts, Prussian blue is found concentrated to some extent in the lumen of the short terminal portion of the tubule and duct. This segment, although short, is cytologically very similar to the terminal portion of the distal convolution and duct as seen in the kidney of higher vertebrates.

SUMMARY

1. A study of the excretion of iron by the vertebrate kidney has been made. After fixation, Prussian blue is found in the form of fine particles in the cells and lumen of a definitely limited portion of the proximal convolution in the rat's kidney in contrast to a more uniform distribution throughout this convolution in lower vertebrates.

2. Iron is retained in the cells of the tubule for at least 4 days. Such retention occurs presumably only after the regulation for physiological excess has occurred. Its active excretion and absorption seem definitely related to its retention only as regards reciprocal loci.

3. In the kidney of one rat the tubules had become in part necrotic. The site and extent of the injury were sharply localized in the epithelium of the first two-thirds of the proximal convolution. The distal third of this convolution and the remainder of the tubule seemed normal. Evidence of functional activity at the time of fixation was manifest in the relatively uninjured portions.

4. Urethane increases the apparent content of iron in the cells of the proximal convolution of the tubule in the frog's kidney by approximately 50 per cent.

5. In the kidney of vertebrates the proximal convolution is primarily secretory and the distal convolution resorptive. The thin segment (mammal) and the intermediate segment (lower vertebrates) are apparently regulatory with reference to the content of fluid admitted by the glomerulus and that admitted by the cells of the proximal convolution and as both fluids in the lumen of the latter are related to absorptive processes occurring in the distal convolution.

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PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of tubules isolated from the kidneys of the rat, pigeon, horned toad, turtle, frog, goldfish, marine fish, earthworm, and marine worm. Figures 4 to 13, $\times 6$, except figure 9, $\times 14$. Figures 14 to 17, $\times 28$. Figures 4 to 11 of vertebrate tubules, photographed at identical magnifications, are for comparison. Figures 9, 10, on invertebrate nephridia, are also for comparison with the vertebrate tubule. Certain details of their structure are shown in figures 18, $\times 280$, and 19, $\times 150$.

4 An entire proximal convolution of the tubule in the rat's kidney.

5 Similar convolution of the tubule in the pigeon's kidney.

6 Two tubules from the horned toad's kidney. The intermediate segment is bracketed.

7 Eight tubules from the turtle's kidney. The intermediate segment of the central tubule is bracketed.

8 Two tubules from the frog's kidney. The intermediate segment is bracketed.

9 Three tubules from the goldfish's kidney with bracketed intermediate segment.

10 Two tubules from the glomerular kidney of the marine fish, *Fundulus*. The bracketed portion is the functional equivalent of the distal convolution in the kidney of the higher vertebrates.

11 Two aglomerular tubules from the aglomerular kidney of the marine fish, *Opsanus tau* (toad fish). Bracketed portion marks the extent of the distal or duct portion.

12 Nephridium of the earthworm.

13 Nephridium of the marine worm, *Nereis*.

14 and 15 A tubule and collecting duct, respectively, from the kidney of the goldfish. Note the central, dark streak in the lumen of the distal convolution and collecting duct, showing concentrated Prussian blue.

16 A nephridium of the earthworm, showing in the lumen of its distal portion concentrated blue. Note faint lumen indicative of unconcentrated Prussian blue in the loop at E.

17 A nephridium of the marine worm, *Nereis*, showing in the more compact left half of the structure, faint, spiral lines of concentrated Prussian blue. This part of the nephridium is the functional equivalent of the distal convolution of the vertebrate tubule.

18 Transection of the nephridium of an earthworm, showing concentrated Prussian blue in the lumen of its distal portion, *B*, and in the bladder, *A*, by the mass to the left of the numeral. Regions of the nephridium designated by letters in figure 16, are similarly designated in this figure.

19 Section of a nephridium of *Nereis*, showing the upper proximal and the lower distal portion with concentrated Prussian blue indicated in the lumen of the latter.

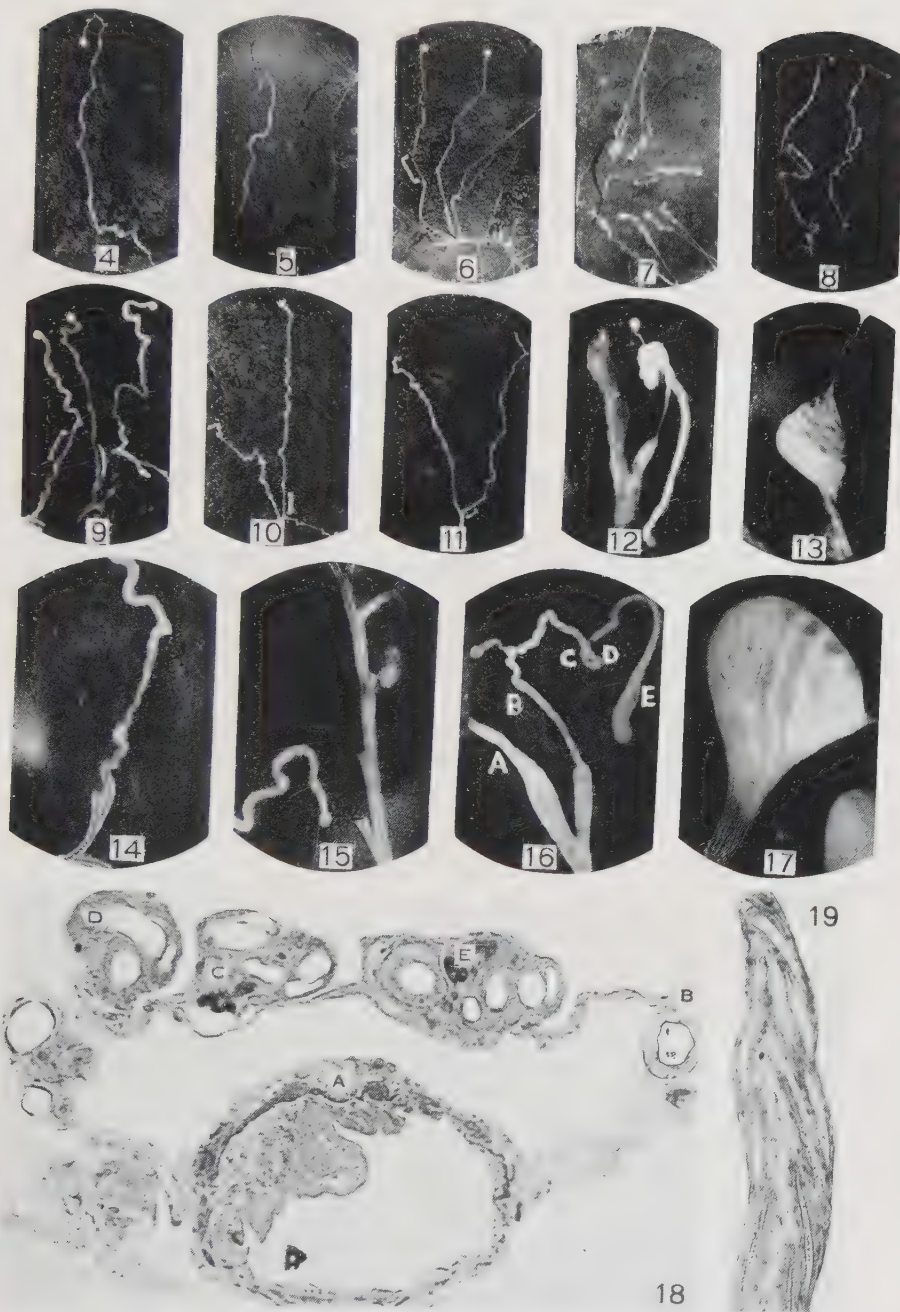


PLATE 2

EXPLANATION OF FIGURES

Photomicrographs of sections of the kidney of the frog, turtle, pigeon, and rat.
× 415.

20 and 21 Sections of a frog's kidney photographed at different magnifications (fig. 21, × 240), showing concentrated Prussian blue in the lumen of the distal convolution—section above the glomerulus and to the left of Z.

22 Section of a turtle's kidney, showing concentrated Prussian blue in the lumen of the distal convolution (center). Note proximal convolutions around the distal.

23 Section of a pigeon's kidney, showing near the top and bottom of the picture, transections, and at the lower center, a longitudinal section of distal convolutions containing concentrated Prussian blue in their lumina.

24 Section through a part of the cortex of the rat's kidney, showing at the center a transection of a distal convolution containing concentrated Prussian blue.

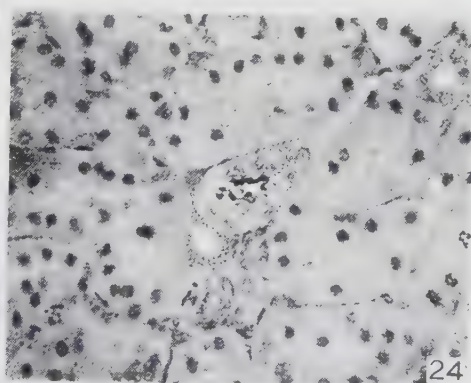
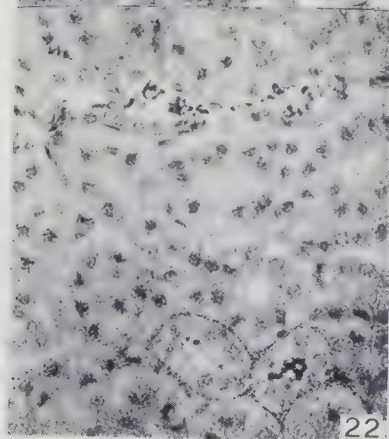
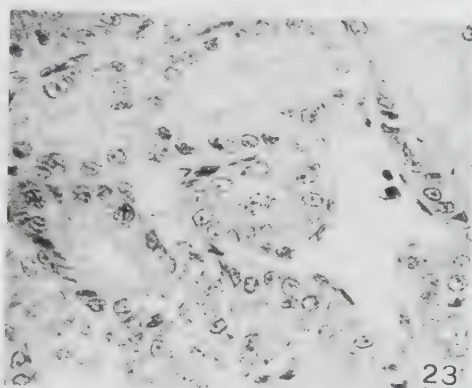
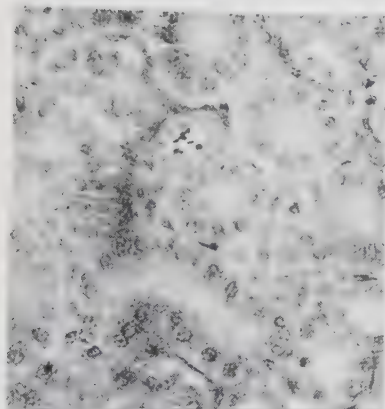
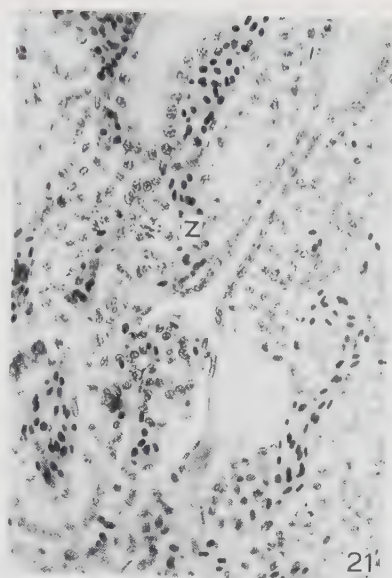
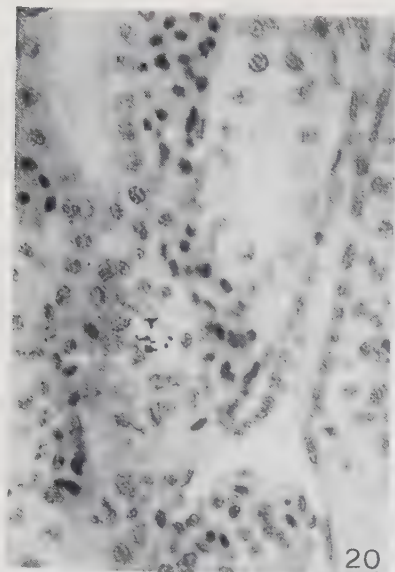


PLATE 3

EXPLANATION OF FIGURES

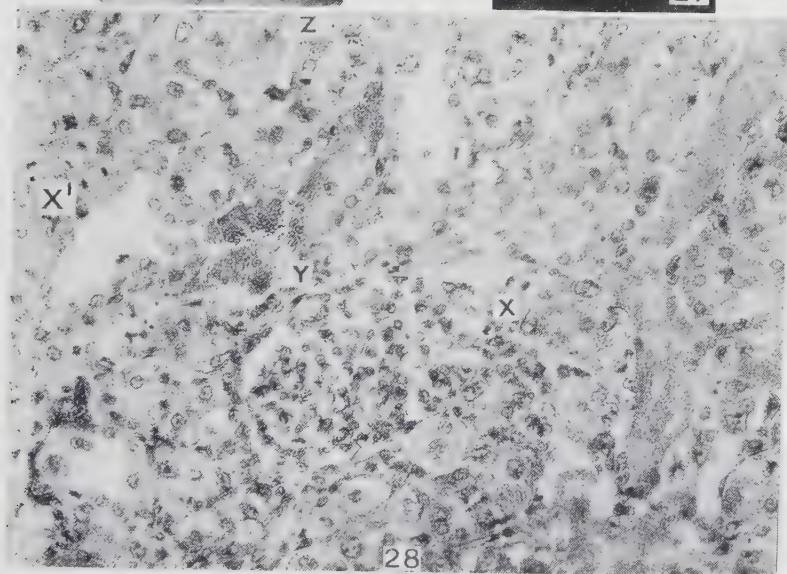
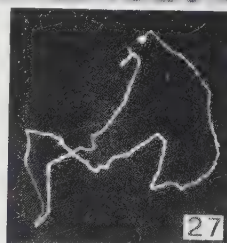
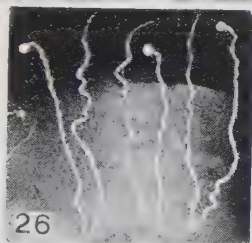
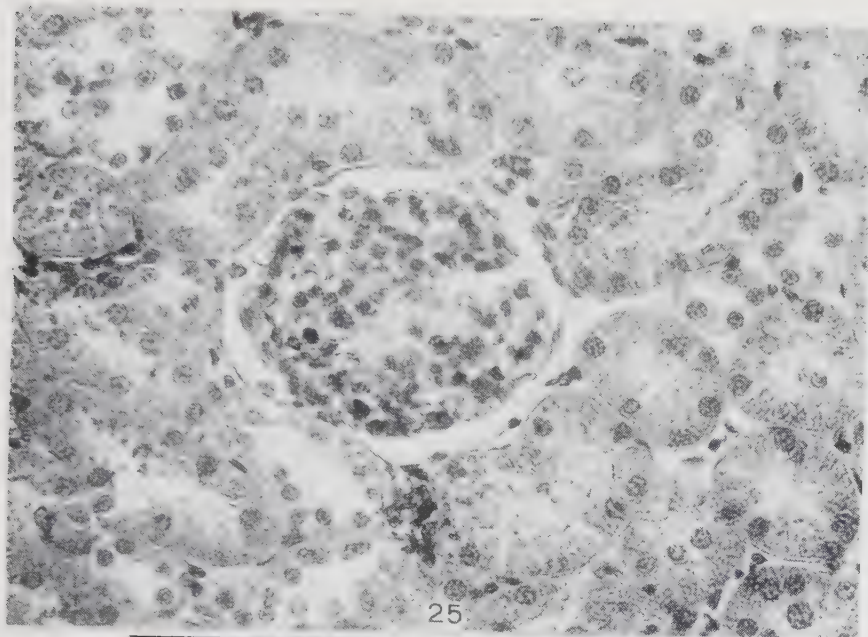
Photomicrographs of sections of the cortex and of isolated tubules from the rat's kidney.

25 Section showing normal proximal convolutions and a glomerulus. Sections of distal convolutions are seen, lower center, bordering on the glomerular capsule and at the left of the numeral, also in the extreme upper left corner. $\times 480$.

26 Three normal proximal convolutions. These were obtained from the macerated half of the kidney, a section of which is shown in figure 25.

27 An entire renal unit which had been injured in the first two-thirds of the proximal convolution. The site of injury is indicated morphologically in the figure, in the first half of this convolution by its smaller diameter and irregular contour. $\times 6$.

28 Section of a portion of the cortex of the injured kidney of the rat, the entire tubule of which is shown in figure 27. Mitotic figures are designated by *X* and *X'* and a section of a proximal convolution by *Y*. The dark appearance of the latter is owing to the presence of fine particles of Prussian blue. A transection of a distal convolution is designated at the right of *Z* and a longitudinal section of a duct is shown at the right of the transection. Note the difference in the size of the black particles present in certain cells of these two sections as compared with the section designated by *Y*.



ON THE FIGURATIVE ELEMENTS OF THE CIRCULATING BLOOD OF SOME BRAZILIAN FLUVIAL TELEOSTEANS

I. ERYTHROCYTES: NORMAL AND ANUCLEATED FORMS, YOUNG FORMS, AND INVOLUTIVE FORMS

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NINE FIGURES

METHODS

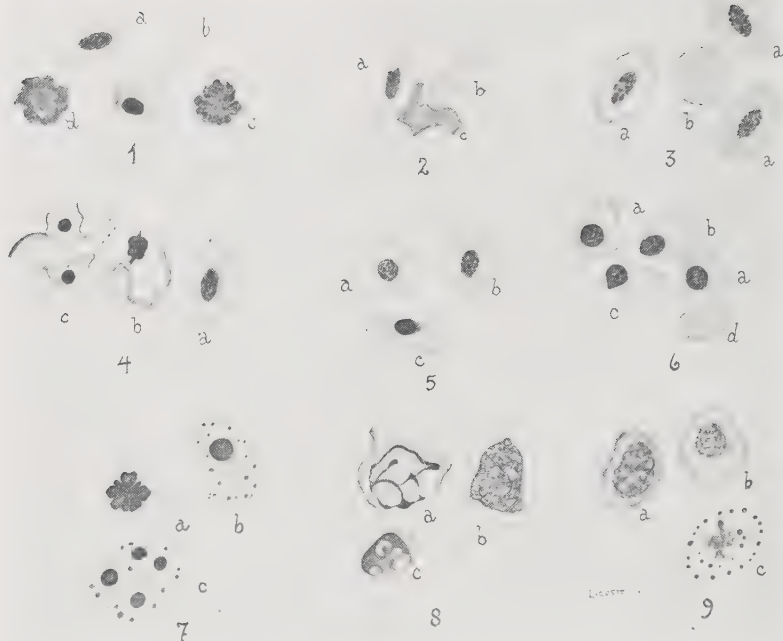
We are resuming herewith our researches on the erythrocytes of different teleostean families (Characidae, Loricariidae, Siluridae, Gymnotidae, and Poeciliidae), indigenous to rivers of the State of São Paulo. Twenty-three distinct species have been observed and whenever we could, we examined numerous individuals of the same species. We are including also a study of *Carassius auratus*, an imported species belonging to the family of the Cyprinidae, which was preserved in an aquarium.

In every case the blood was extracted from the gills of the fishes and the blood films were treated by the usual hematological methods.

Blood trypanosomes were found in nine species already described by Fonseca and Vaz(4, 5) in their parasitological studies on Brazilian fluvial fishes.

NORMAL FORMS

The erythrocytes in the Characidae have the classical form of ellipsoidal discs (figs. 1, *a*; 2, *a*; 3, *a*; 4, *a*); however, in the Nematognatha (Loricariidae and Siluridae) the form is frequently round and rather circular (figs. 5, *a* and *b*). Up to



All figures were drawn under the same magnification (1500 diameters), from blood films treated by Leishman and Pappenheim's stains.

Fig. 1 *a*, a normal ellipsoidal erythrocyte; *b* and *c*, erythrocytolysis (normal involution); *d*, a residual nucleus after plasmolysis (*Acestrorrhampus* sp., Characidae).

Fig. 2 *a*, a normal erythrocyte; *b* and *c*, two anucleated particles and residual nucleus from degenerated erythrocyte (*Leporinus* sp., Piava, Characidae).

Fig. 3 *a*, normal ellipsoidal erythrocytes; *b*, a round red corpuscle without the nucleus (*Piaractus brachypomus*, Characidae).

Fig. 4 *a*, a normal form; *b* and *c*, atypical dendritic forms of erythrocytheresis (*Acestrorrhampus* sp., Characidae).

Fig. 5 *a* and *b*, circular and round erythrocytes; *c*, segmented form with the expulsion of the nucleus (*Oxyloricaria* sp., Nematognatha).

Fig. 6 *a*, normal forms of the *Phalloceros caudimaculatus* (Poeciliidae); *b*, *c*, three periods of expulsion of entire nucleus.

Fig. 7 *a*, *b*, *c*, three appearances of abnormal involution: the increased size and the karyorhexis of the erythrocytes (*P. brachypomus*, Characidae).

Fig. 8 *a*, associated karyolysis and rhexis; *b*, karyolysis of the increased forms (*P. brachypomus*); *c*, lysis after pycnosis of the nucleus in a microcytic form (*Leporinus* sp., Piava).

Fig. 9 *a* and *b*, a basophilic and polychromatophilic erythroblasts; *c*, rhexis of a free nucleus (*Leporinus* sp., Piabussu).

this time so many exceptions to the classical form of the teleostean erythrocyte have never been reported in the literature.

Further, in the Petromyzontes whose red corpuscles are classically round (Wagner(23), Wharton Jones(24), Gulliver (8), Thompson(22), Gage(22), Giglio-Tos(6), etc.), we have observed the same characteristics as in the Nematognatha.

Again, in the Gymnotidae, of which unfortunately we could study only one example (*Carapus fasciatus*), the red cells presented a shape tending to the round form. The same may be said of the Poeciliidae. However, in the latter family the deformed red corpuscles are very numerous, since it appears that such a poikilocytosis is a usual characteristic of viviparous species (*Phalloceros caudimaculatus*, fig. 6, a).

The nucleus of the erythrocytes of the teleosteans have almost always a form which is in accordance with the cell's shape, except in Loricariidae, where so frequently some nuclei present irregularities (triangular, trilobated, and segmented), that we may conclude, perhaps, that they are characteristic of this family.

The size of the normocytes is variable not only in single individuals, but also in the different species and families; however, the dimensions approximate those given in the hematological literature for the Teleostei.

In contrast to individuals with large red cells (some Loricariidae) we find others having proportionately smaller elements (Poeciliidae and Gymnotidae). In the Loricariidae the dimensions may reach as high as $15 \times 12 \mu$, while in the *Carapus fasciatus* (Gymnotidae) they average $9.5 \times 7.5 \mu$, and in the *Phalloceros caudimaculatus* (Poeciliidae), $8 \times 7 \mu$. In the scaly fishes (Characidae) the diameters in the ellipsoidal erythrocytes are frequently about $13 \times 8 \mu$; near the latter are the figures for *Carassius auratus*.

Therefore, we may conclude in general that fishes, due to the size of their red blood cells, may be placed between most of the birds (which, except mammals, have the smallest red cells among the vertebrates) and the majority of the reptiles.

In fishes, only the Dipnoi (Lepidosiren) approach the amphibians in dimensions of their red cells.

In this respect, all the literature consulted by us is accordingly unanimous, from the classical works of Gulliver and Milne Edwards, to the most recent researches of Jolly(9), and Babudieri(1).

NON-NUCLEATED DISCS

Among the normocytes of our Teleostei, especially in the *P. caudimaculatus*, we find relatively numerous non-nucleated discs; they are less frequent in the Characidae and very rare in the Nematognatha.

We also observed, agreeing with Jolly and Babudieri (loc. cit.), that the discs without nucleus are more rare in fishes than in amphibians and reptiles, even considering the relatively high percentage rate found by us in *P. caudimaculatus* (0.5 per cent).

The forms without nucleus may originate by segmentation (fig. 1, *b*): from a normocyte comes a nucleated microcyte and an 'erythroplastid' (Emmel(3)). This process of cellular merotomia observed by us especially in the Characidae, and in *Batrachoseps attenuatus*, *Petromyzon*, and some Amphibians by Rollet (1862), Stricker(21), Eisen(2), Giglio-Tos (7), Maurer(15), Schaffer(20), Emmel(3), Komocki(14), etc., is not the only method by which cells may give rise to non-nucleated elements.

Thus, the non-nucleated discs may proceed from erythrocytes which expel the entire nucleus (*P. caudimaculatus*), for which process poikilocytosis is in part responsible (figs. 6, *b*, *c*, and *d*). Komocki(14) has recently described the same process in the *Batrachoseps attenuatus*.

A third source of non-nucleated elements was observed in some Characidae (*Piaractus brachypomus*, *Leporinus* (several species)), where sometimes the erythrocytes eliminate the nucleus by either lysis or rhexis, or suffer karyocytoplasmic disintegration delivering non-nucleated particles, 'schistocytes' (fig. 2, *b* and *c*). These non-nucleated particles

have irregular outline and size, but the non-nucleated discs which originate either by merotomia of the cytoplasm or by integral expulsion of the nucleus, are circular and orthochromic seeming to have the functional value of normocytes (fig. 3, *b*).

YOUNG AND INVOLUTIVE FORMS

In some specimens, the basophilic, polychromatophilic (fig. 9, *a* and *b*), and orthochromatic erythroblasts are very abundant; in this case they are the products of regeneration due to effects of abnormal agents, which are not well defined. It also happens that in these individuals we observe the correlative fact of great numbers of degenerated erythrocytic forms. At other times, the young forms are more rare in the circulating blood; in this case, as most of our material has been collected at the end of spring, its existence may be considered as normal, due, perhaps, to the physiologic influx of blood renovation occurring in the springtime. We never observe mitosis in ultimate erythroblasts.

Very opportune, in our material, is the analysis of the involutive forms, the so-called 'atypical erythrocytes.' We may observe several phases of the terminal cycle of the circulating erythrocytes in the Brazilian fluvial teleosts. The process which occurs more frequently has been described as a common process in vertebrates with nucleated red cells (Rawitz (18, 19), Jordan-Flippin(12), Jordan(10), Komocki(13)). The degenerating red cells increase their volume, take the round outline, and progressively lose their typical structure. The nucleus becomes either globulous or starry, with the chromatin palely homogeneous or roughly reticulated. The cytoplasm dissolves itself soon, there is a total plasmolysis, and the residual nucleus remains free (fig. 1, *c* and *d*).

At other times, the nucleus increases its volume, vacuoles appear in its interior and it acquires an alveolar structure; the cytoplasm may be reduced to a simple border, or it may be hidden under the augmented nucleus, and sometimes may even disappear. This process is less frequent (fig. 8, *b* and *c*).

Still less frequent are the figures of erythrocathe-
resis, which begin by nuclear pycnosis and finish by karyolysis or
by isolated or associated karyorhexis. In all these cases there
is an increase of the cell's shape, the cytoplasm remaining
lightly colored. Here the aspects of degeneration are multiple
and are distinguished by the variety of the nuclear fate.

Sometimes the nucleus is only a pycnotic mass (figs. 4, *b*;
5, *c*), at other times it takes the form of a thick anastomotic
net, which eventually fragments into granular corpuscles
(fig. 8, *a*). It again takes the form of a trifolium which
finally breaks into unequal chromatic corpuscles, which then
subdivide into smaller granules which are spread through
the discoplasm (fig. 7, *a* and *c*); in other cases the pycnotic
nucleus divides into two identical pycnotic masses (fig. 4, *c*),
or the spherical nucleus expels round granules of chromatin
(fig. 7, *b*), or maintaining its normal shape emits dehiscent
chromatic particles, etc. The particles and the granules,
resulting from the degenerated nucleus, are frequently seen
liberated after the death of the cells (fig. 9, *c*).

All those multiple transformations of the erythrocytes can
be very intensive in one or another species (*Piaractus brachy-*
pomus, *Leporinus* sp., etc.); in these individuals the erythro-
cathe-
resis is quantitatively and qualitatively unusual.

These atypical forms resemble those described by Nigrelli
(16) in *Triturus viridescens* which is infested by trypano-
somes. However, it is very difficult to point out the cause
which so intensely affects these red cells.

In the case of abnormal erythrocathe-
resis, there is
another cause—perhaps the toxic influences of the frequent
parasites in fishes of the Brazilian rivers. If the last hy-
pothesis is right, the trypanosomiasis is not the only cause,
since of the nine species carrying flagellates, only one was
shown to be affected by that intensive process; moreover, in
two species where the erythrocathe-
resis was more intense
and complicated, the trypanosomes did not appear.

Therefore, we must consider other agents: protozoa,
trematodes, nematodes, etc. But we find it opportune to con-

clude that not always are the common parasites of our fishes pathogenic.

Finally, we wish also to call attention to inanition, which has recently been noted by Jordan(11) in *T. viridescens*. This author reproduced by inanition the figures described by Nigrelli. We also obtained increase in number of the usual involutive forms by submitting to inanition some individuals of *Astyanax fasciatus*.

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THE PRENATAL GROWTH OF THE CAT

III. THE GROWTH IN LENGTH OF THE TWO EXTREMITIES AND OF THEIR PARTS

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FOUR FIGURES

The linear growth of the fore limb, the hind limb, and the three major divisions of each appendage will be presented in this paper. All of the measurements were made on the fore limbs of the 264 specimens, but in nine of the smallest fetuses the hind limbs were so poorly developed that only the length of the entire extremity could be determined accurately. All of the measurements of the hind limb were made on the remaining 255 specimens.

The four extremities were removed, as described in the first paper of this series, for weighing the entire extremity. After weighing, the following measurements were made. All measurements were made and recorded for both the right and the left sides, and the average of the two is meant whenever it is not otherwise stated. The arm was measured from the head of the humerus to the tip of the elbow. Next, the length from the tip of the elbow to the tip of the toes was measured. The length of the forefoot was measured from the tip of the toes to the dermal pad on the ulnar side of the wrist. This pad, according to Reighard and Jennings ('01), marks the location of the pisiform bone, and it seemed to be the best external landmark for separating the forefoot from the forearm. In a few of the smallest specimens there was a second smaller pad proximal to this pad. The difference between these last two measurements gives the length of the forearm, and the sum of the shoulder-elbow and the elbow-toe

measurements gives the total length of the fore limb. These specimens had been formalin hardened, so that it was very difficult to straighten the appendages to make a direct measurement of the total length, and so this method is more accurate than a direct measurement of the total limb length.

The hind limb was measured in a similar manner. The length of the thigh was measured from the proximal part of the head of the femur to the patella. The second measurement was made from the patella to the tip of the toes, and the third was the length of the foot from the tip of the longest toe to the proximal side of the calcaneus. The total length of the limb was the sum of the first two measurements and the length of the leg is the difference between the second and the third measurements. The hind limbs were more easily and more accurately removed from the body, but the fore limbs were easier to measure accurately. The hindfoot was flexed on the leg very frequently, so that it was hard to get the knee-toe measurement accurately, and it will be shown later that the leg length is the most variable dimension in this series of measurements. The foot was gently but firmly straightened in making the knee-toe measurement.

The author wishes to gratefully acknowledge the assistance of Dr. John M. Aikman in making the measurements on the first 137 specimens.

FORE LIMBS

The lengths of the fore limb and of its three segments plotted on body length (nose-anus) are shown in figure 1. The dots represent the average length of the two fore limbs of the males, and the circles the average of the two limbs of the females. The distribution of the cases for the lengths of the arm, forearm, and forefoot is very similar to that for the fore limb, with no apparent sex difference in any of the measurements, and so only the curves for the three segments are shown in this figure. This facilitates the comparison of the relative lengths of the parts and of the entire appendage.

The empirical formulae from which all four curves were drawn are:

Fore limb,	$Y = 0.453 X - 7.6$
Arm,	$Y = 0.1735 X - 3.2$
Forearm,	$Y = 0.16 X - 3.3$
Forefoot,	$Y = 0.119 X - 1.3$

In all of these formulae Y represents the length of the part measured in millimeters, and X the body, or nose-anus, length also measured in millimeters, and plotted as the abscissae in the figure. All of these formulae are valid between 30 and 200 mm. of body length. The four curves of absolute growth

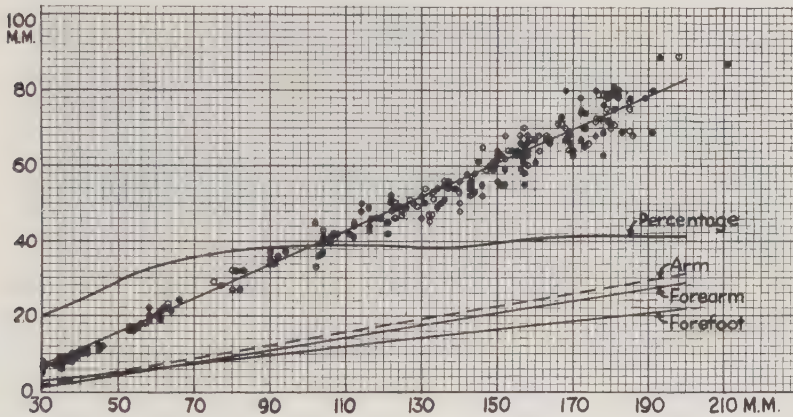


Fig. 1 The growth of the fore limb plotted on body length in the upper curve with the dots representing the males, and the circles, the females. Curves are shown for the three segments, but the individual cases are not given. The values for the percentage curve are in the left margin, although marked 'mm.' The formulae for the four curves of linear growth are given in the text.

are all straight-line curves, or they exhibit a constant rate of increase throughout this period.

The curve in this figure marked 'percentage' represents the total length of the fore limb expressed as a percentage of the body length. The figures in the left margin marked 'millimeters' serve not only to show the actual lengths, but also the percentages for this curve. On the preliminary chart all of the individual percentages were plotted and a line was then drawn through the averages for each 10 mm. in-

crease in body length, and this line only was transferred to this figure. The distribution of the percentages about this line was fully as close as the cases about the curve in figure 1. This curve shows that the length of the fore limb increases from about 20 per cent of the body length to slightly over 40 per cent, or the length of the fore limb grows about twice as fast as the body length during the fetal period.

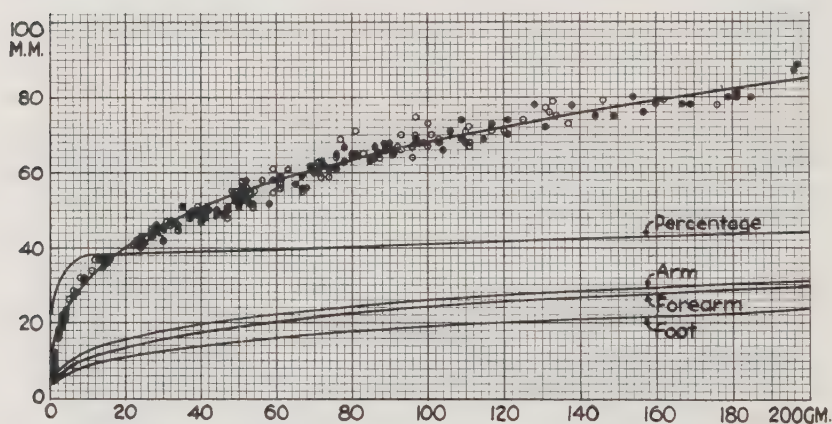


Fig. 2 The growth of the fore limb and its segments plotted on body weight. The upper curve gives the total length with the dots representing the males, and the circles, the females. The individual cases are not shown for the three segments of the appendage. Formulae for all four curves are given in the text. The ordinates in the left margin, although marked 'mm.,' give the correct values for the percentage curve.

Figure 1 and the computed lengths show that the forefoot is the longest part of the fore limb in the smallest specimens. It is surpassed first in length by the arm at 40 mm. of body length, and at a body length of 50 mm. and over it is the shortest of the three divisions. The entire fore limb increases about 14 times. The forefoot increases a little less than 10 times, the arm about 16 times, and the forearm about 19 times.

Figure 2 shows the lengths in millimeters of the fore limbs and the three parts plotted on body weight in grams. The upper curve with the dots representing the males and the

circles the females shows the linear growth of the fore limb. The lower three curves represent the linear growth of the arm, forearm, and forefoot, and, as in the preceding figure, the cases for these are not included in this figure. The curve marked 'percentage' represents the average percentage of the length of the fore limb of the nose-anus length plotted on body weight. It was drawn in a manner similar to the curve in figure 1. The distribution of the cases on the preliminary chart is possibly a little closer in this case, especially for the larger specimens. The figures in the left margin serve for percentage values for this curve as well as millimeters for the curves of linear growth.

It is interesting to note that in the formulae for the linear growth of the fore limb and for its three parts and likewise, as will be shown later, for the hind limb and its three segments, the increase in length is a function of the cube root of the body weight. The formulae from which the four curves in this figure are drawn are:

Fore limb,	$Y = 14.5 \sqrt[3]{X}$
Arm,	$Y = 5.8 \sqrt[3]{X} - 0.015 X$
Forearm,	$Y = 5.2 \sqrt[3]{X} - 0.5$
Forefoot,	$Y = 4 \sqrt[3]{X}$

In all of these formulae Y represents the length of the extremity or its part measured in millimeters, and X, the body weight in grams from 1 to 200 gm. of body weight.

A comparison of the distribution of the cases in the curve of the fore limb in this figure and in figure 1 shows that there is a massing of the cases at the beginning of the curve in this figure, while in figure 1, the cases are crowded toward the end of the curve. In other words, the body length increases more rapidly at first than the body weight, as has been shown in figure 1 of the first paper of this series. The curve representing the growth of the forefoot does not cross the other two curves as it did in figure 1. This is due to this crowding of the cases at first and also because these formulae begin at 1 gm. of body weight, which excludes many of the smaller specimens. The formulae will not fit the cases below 1 gm. The curves in figure 1 are more accurate for the smaller specimens.

The percentage increments of the fore limb, and of its parts computed on body weight are larger at first and decrease to smaller values than the corresponding increments computed for body lengths. This is as would be expected, since the linear dimensions increase more rapidly at first than the weight.

To derive the empirical formulae in this and the preceding papers of this series, the dimensions were plotted on a field graph; next, the average of the dimension and of the abscissa were determined for each 10-gm. increase in weight or each 10-mm. increase in body length. A smooth curve was drawn through as many of these average points as possible and an empirical formula developed to fit the curve. The formula was solved for each 10 units of the abscissa and plotted on the final chart, and through these points the final curve was drawn. To give an idea of the accuracy with which these curves in figures 1 and 2 and also in the two following figures fit the cases, the difference between the observed length and the calculated length for each 10 mm. or 10 gm. increase in the abscissae was determined. The table giving the average linear differences for each curve and also the average percentage differences for each curve is omitted. The curve for the forearm plotted on body length has the greatest average percentage difference (5.04), and it is the only one of the six curves for the fore limbs which has a percentage difference exceeding 5 per cent. All three curves plotted on body length are not as well fitted to the cases as the three plotted on body weight. The smallest percentage difference of 1.65 per cent is found in the curve of fore limb length on body weight.

The cat fetus resembles the human fetus in the straight-line curves of the upper extremity and its parts plotted on body length as given by Scammon and Calkins ('29) and others, and the increasing percentage of the body length resembles the changes in the percentages of the human upper extremity. The percentage values given by Scammon and Calkins ('29) are not comparable with the percentages given here, for these percentages are based on nose-anus length, and not crown-

rump or crown-heel lengths. No attempt will be made to discuss the rather extensive literature on the growth of the linear dimensions of the extremities of the human fetus, for this has been so well done by Scammon and Calkins ('29). Schultz ('26) finds a decrease in the growth of the human extremities in relation to the trunk height from the sixth to the tenth fetal month and similar changes in apes and monkeys. The first part of his percentage curve rises as do the

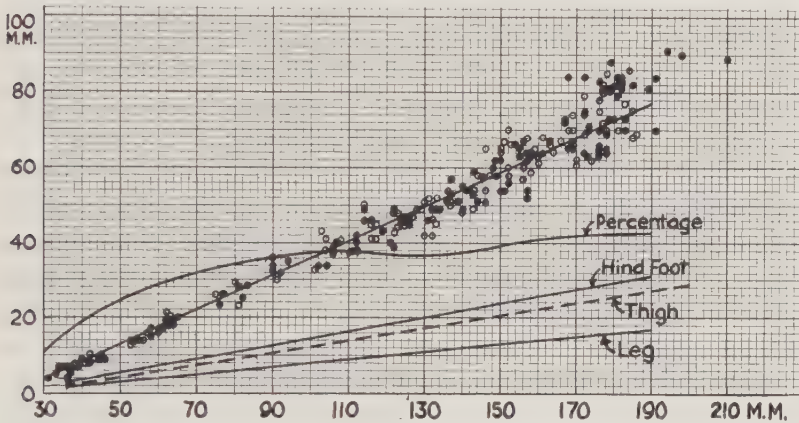


Fig. 3 Growth of the hind limb and its segments plotted on body length is shown in the upper curve. The dots represent males, and the circles, females. The individual cases are not shown for the three segments of the extremity. Formulae for the four curves are given in the text. The ordinates in the left margin give the values for the percentage curve as well as for the other curves.

percentage curves for the cat extremities. His curve rises more rapidly at first and the dip toward the end is much more pronounced than in the cat fetus. In general, the linear growth of the fore limbs of the cat fetus resembles that found in man and monkeys.

HIND LIMBS

The linear growth of the entire hind limb plotted on body length is shown in the upper curve in figure 3. The individual dimensions are shown as dots for the males and circles for the females. The three lowest curves, with the individual

cases omitted, represent the growth in length of the three parts of the hind limb. The formulae for all four curves are given below.

Hind limb,	$Y = 0.458 X - 9.74$, from 30 to 190 mm. of body length
Thigh,	$Y = 0.165 X - 4.025$, from 35 to 200 mm. of body length
Leg,	$Y = 0.1 X - 2.1$, from 36 to 190 mm. of body length
Hindfoot,	$Y = 0.186 X - 3.70$, from 36 to 190 mm. of body length

In all of these formulae Y represents the length of the limb, or its part, in millimeters, and X , the body length (nose-anus) also measured in millimeters. The formulae for the parts of the extremity begin at either 35 or 36 mm. of body length, for, as has been previously stated, the hind limbs of the smallest specimens were not sufficiently developed to permit the measurement of the parts of the appendage with any degree of accuracy. This slower development of the hind limb as well as its shorter length and lighter weight (Latimer and Aikman, '31) illustrates the law of developmental direction.

The distribution of the cases for the hind limb, the leg, and the hindfoot differs from the lengths of the other extremity and the other parts plotted on body length in that these dimensions in the newborn cats are longer and so are above the curve drawn for the fetuses. In figure 3 all of the cases above 71 mm. of length represent the length of the hind limb of newborn cats. This greater length is apparent in the preliminary charts for the growth of the leg and of the hindfoot, but not for the thigh. This increase in the lengths of these dimensions is not quite as evident when the body weights are used as abscissae. In figure 4 the cases between 121 and 160 gm. of body weight are newborn cats, and they show a rather sudden increase over the lengths of the fetuses just less than 121 gm. of body weight. All of the fetuses weighed 121 gm. or less. A possible explanation of this increase is the straightening of the ankle joint at birth. It has been stated above that it was difficult to straighten this joint in the fetuses in order to get good measurements of these parts. This would explain the increase in the leg and the entire extremity, but it is not a satisfactory explanation for this increase in the foot length.

The percentage curve in this figure represents the lengths of the hind limbs reduced to percentages of the body lengths. The figures in the left margin represent the percentage values as well as millimeters of length. Thus the hind limb forms but about 10 per cent of the body length at first and increases to a little over 40 per cent. There is a marked similarity between this curve and the percentage curve of the fore limb shown in figure 1. The greatest difference is at first, for the fore limb starts with about twice the percentage length of the hind limb. This is due, of course, to the more precocious development of the fore limb.

The foot is the longest segment of each extremity at first. It has been shown that the forefoot is rather quickly surpassed in length by the other two segments of the fore limb, but it will be seen from figure 3 that the hindfoot remains the longest segment of the hind limb throughout the entire fetal period and also in the newborn. The leg is the shortest part and the thigh intermediate throughout the entire period.

Using the computed lengths of the two extremities and their parts, it is readily seen that the entire hind limb as well as each of its parts is shorter at first and increases more in the fetal period than the fore limb or the corresponding parts of the fore limb. The hind limb increases 20.4 times in length, or nearly one and a half times the similar increase of the fore limb. The thigh increases more than any of the other parts or the two extremities. It increases over 31 times, or nearly twice the increase of the arm. The leg increases 19.9 times, which is very nearly the same as the total increase for the forearm. The hindfoot increases 17.8 times, or about one and eight-tenths times the total increase for the forefoot. The larger percentage increments (not shown) throughout the entire period for the hind limb and its segments compared to the fore limb and its homologous segments also evidence the greater growth of the hind limb and its parts. Further discussion of the relative lengths will be given later.

The upper curve in figure 4 represents the length in millimeters of the hind limb plotted on body weight in grams, with

the males shown as dots and the females as circles. The three lowest curves represent the linear growth of the three segments of this extremity. As in the preceding figures, the cases are not shown for these, as the distribution of the individual cases, as seen in the preliminary chart, is very similar to that for the entire extremity.

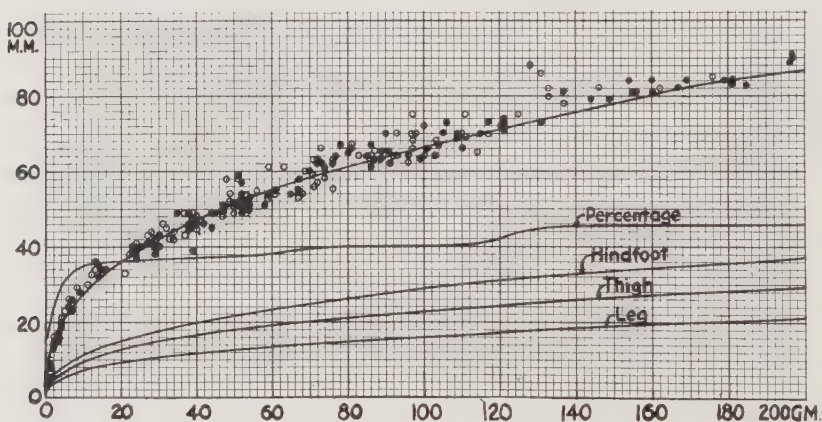


Fig. 4 Growth of the hind limb plotted on body weight is shown in the upper curve with the dots representing the males, and the circles, the females. Individual cases are not given with the curves for the segments of the extremity. Formulae for the four curves are given in the text. The ordinates in the left margin give the correct value for the percentage curve as well as for the other curves.

The empirical formulae for these four curves, like the formulae for the four curves in figure 2, are all functions of the cube root of the body weight. The formulae from which the four curves were drawn are as follows:

Hind limb,	$Y = 16 \sqrt[3]{X} - 7.00$, from 3 to 200 grams of body weight
Thigh,	$Y = 5.2 \sqrt[3]{X} - 1.00$, from 2 to 200 grams of body weight
Leg,	$Y = 3.1 \sqrt[3]{X} + 0.016X$, from 3 to 200 grams of body weight
Hindfoot,	$Y = 7.1 \sqrt[3]{X} - 4.00$, from 3 to 200 grams of body weight

In all of the above formulae, Y represents the length in millimeters of the extremity or its part and X represents the body weight in grams. In every case the average percentage deviation of the calculated from the observed length is less for the curves plotted on body weight than for those having

body length as abscissae. The leg has the greatest average percentage deviation in both sets of eight curves (7.07 per cent for body length and 4.43 per cent for body weight). All but two of these curves (leg and forearm on body length) have an average percentage deviation less than 5 per cent.

The lengths of the hind limb reduced to percentages of the total body length are shown in the percentage curve in figure 4. The numbers in the left margin, although marked millimeters, give the correct percentage values for this curve. This curve rises more rapidly at first than the percentage curve in figure 3 and it shows no decrease, as is evident at about 130 mm. of body length in figure 3.

In general, the growth of the hind limb resembles the growth of the lower extremity of the human fetus in that all curves based on body length are straight lines (Scammon and Calkins, '29). Likewise, the lower extremity of the human fetus, as we have shown for the hind limb of the cat fetus, has a smaller initial length and increases more during this period. Both extremities become increasingly heavier in proportion to their length, with the greatest increase in the hind limbs. The weight in grams for each centimeter of length of the appendage was determined for both extremities for each 10-gram interval of body weight. The fore limb increased from 0.020 to 1.50 gm. per centimeter of length, or 75 times, between 0.3 and 190.0 gm. of body weight, and the similar values for the hind limb are, from 0.0216 to 1.98 gm. per centimeter of length, or an increase of 91.7 times. The marked differences in the growth of the two extremities are the greater growth of the hindfoot compared with the forefoot and the shorter leg compared with the forearm.

Scammon ('30) has shown that the percentage first derivatives for the ponderal growth of both extremities, the hands and the feet of the human fetus, are remarkably constant for the latter part of the period. The first differentials of the eight formulae for linear growth of the extremities and their parts on body weight, given above, were determined for each 10-gm. increase in body weight. These differentials were

then reduced to percentages of the length in centimeters of the extremity, or part, for that body weight, or $\left(\frac{\delta L}{\delta W}\right)_{L \text{ (cm.)}}^{100}$. The resulting values for the gain in length per unit of body weight were also very similar and especially for the later part of the period. The percentage differentials for the fore limb and its parts were more constant than the similar percentage differentials for the hind limb and its three segments. The percentage differentials for the leg length were less than for any of the other seven dimensions.

RELATIVE GROWTH

The percentages of the lengths of the fore limb and of the hind limb with reference to the body length have been shown in figures 1 and 3 plotted on body length and in figures 2 and 4 plotted on body weight. The lengths of the three segments of each appendage were reduced to percentages of the total length of the respective appendage, and plotted on body weight (curves not shown). The percentages of both the arm and forearm rise rapidly at first. The percentage lengths of the forearm in all of the cases below 1 gm. of body weight average 27 per cent. This increases to 33 per cent in the second group of cases and increases but little up to birth, with a slight decrease in the newborns. The curve for the percentages of the arm length rises less than that for the forearm, for it averages 36 per cent for all of the cases below 1 gm. of body weight and after a rapid though brief rise it averages 37.96 per cent from 1 to 120 gm. of body weight. It drops 1 per cent in the newborn cats just as the curve for the forearm does. The percentage length of the forefoot decreases rapidly between the first and second groups, or from an average of 36 per cent to 29 per cent in the second group, and then remains nearly constant.

The three parts of the hind limb are not as constant in their percentages as are the parts of the fore limb. The percentages of the hindfoot average 47 per cent for the first group, or all of the specimens less than 1 gm. in body

weight. This decreases to 41 per cent in the specimens between 1 and 10 gm. in weight and then slowly increases to its maximum of 43 per cent. The other two segments of the extremity increase at first as did the two proximal segments of the fore limb, but these vary more than the parts of the fore limb. The same percentages were plotted on body length as abscissae, and the resulting curves were practically the same as those plotted on body weight.

The most striking thing shown in these percentages is the relative decrease in the percentage length of the forefeet and the hindfeet and the corresponding increase in the two proximal parts of both extremities. These changes in the early proportions of the extremities together with the failure of the formulae to fit the smaller specimens, which has been mentioned, would suggest that these smaller specimens belong to the embryonic rather than the fetal period, or at least that there is some marked change in the rate of growth between these smallest specimens and the remainder of the fetuses.

INDICES

The ratios of the adjacent segments of the same extremity are shown in columns 2 to 5 of table 1. All of the indices in this table are the averages of the individual indices for the specimens grouped according to body weight as shown in the first column. The number of specimens in the last three body weight groups is much smaller than in any of the others, and hence these indices are less significant. The brachial index ($\frac{\text{forearm} \times 100}{\text{arm}}$) increases rapidly at first, and to a lesser extent this is true of the tibio-femoral index ($\frac{\text{leg} \times 100}{\text{thigh}}$). Both indices increase slowly and somewhat irregularly throughout the entire period, thus showing that the proximal segment of both extremities grows less rapidly than the middle segment. Scammon and Calkins ('29) report a similar condition for the human fetus. They find that the distal segments grow more rapidly than the middle, but this is not true for the cat fetuses, for the third and the fifth columns of this table show that the forefoot-forearm ($\frac{\text{forefoot} \times 100}{\text{forearm}}$) and the

foot-leg ($\frac{\text{hindfoot} \times 100}{\text{leg}}$) indices decrease rapidly at first and the forefoot-forearm index continues to decrease more slowly throughout the entire period.

All of these indices were plotted and the distribution of the cases was very good for all of the indices, except the

TABLE 1

Averages of the individual indices, for each 10 gms. of body weight, of the adjacent segments of the same extremity and of homologous segments of the fore limb and the hind limb

BODY WEIGHT, GRAMS	BRACHIAL	FOREFOOT-FOREARM	TIBIO-FEMORAL	FOOT-LEG	INTER-MEMBRAL	HUMERO-FEMORAL	HAND-FOOT
0.3- 1	74.7	138.8	68.3	221.1	131.7	137.2	94.9
1 - 10	88.9	86.7	70.3	170.1	117.1	127.2	82.8
10 - 20	90.3	82.8	69.3	161.6	107.8	114.2	77.3
20 - 30	88.3	82.5	64.4	181.7	106.6	113.6	73.2
30 - 40	89.4	82.1	65.5	178.0	105.2	112.6	71.5
40 - 50	86.2	86.8	62.3	186.0	103.2	109.5	71.3
50 - 60	88.7	82.1	61.9	191.4	103.6	110.7	68.8
60 - 70	91.8	80.1	65.6	187.1	102.5	110.4	66.9
70 - 80	92.0	78.9	67.3	186.9	100.1	110.3	64.5
80 - 90	90.2	79.9	65.9	190.6	101.3	110.4	64.9
90 -100	93.5	78.3	66.1	187.3	102.4	111.1	66.2
100 -110	90.6	78.6	63.2	192.5	101.6	110.3	64.7
110 -120	93.4	77.7	68.0	186.9	99.8	110.0	63.5
120 -130	96.4	76.9	70.3	180.9	97.7	106.8	63.2
130 -140	95.4	80.9	74.0	171.0	94.9	104.3	63.7
140 -150	95.5	79.3	68.4	183.8	95.0	103.0	62.3
150 -160	95.2	80.0	70.7	177.5	96.3	105.0	63.5
160 -170	92.7	80.7	74.0	173.1	94.3	106.0	62.0
170 -180	93.0	81.5	77.0	172.0	94.5	109.0	62.0
180 -190	95.7	78.3	70.5	181.0	96.3	105.7	62.3
190 -200	97.5	77.0	70.5	181.0	98.5	107.0	63.0

foot-leg index. The curves are omitted to save space. The values for this last index are probably of less significance than the others, due to the very wide variation in the indices for each group.

The last three columns of this table give the average indices for the homologous segments of the fore limb and the hind limb. The general trend of the intermembral index ($\frac{\text{fore limb} \times 100}{\text{hind limb}}$) is similar to that of the human fetus and

the indices are shown in column 6, table 1. The average index for the smallest fetuses is 131.7, and Schultz ('26) gives a percentage of 132.7 for the human fetus at 9 weeks. He finds that the upper extremity is longer than the lower, except at 34 weeks of age. The fore limb of the cat is shorter for all body weights above 113 gm. The index of 94.2 per cent given by Scammon and Calkins ('29) for the newborn human is similar to that found for the newborn cat. Their values at first are lower than those given by Schultz or the indices for the cat fetus.

The ponderal index ($\frac{w. \text{ fore limb} \times 100}{w. \text{ hind limb}}$) was computed from table 3 of the first paper of this series and, unlike the intermembral index, it increases from 125 at 0.3 gm. of body weight to its maximum of 170.2 at a body weight of 1 gm. and then it decreases to 74.4 in the largest specimens.

Next to the last column in table 1 gives the humero-femoral index ($\frac{\text{arm} \times 100}{\text{thigh}}$) and it shows that the arm is longer than the thigh throughout the entire period. The last column giving the hand-foot index ($\frac{\text{forefoot} \times 100}{\text{hindfoot}}$) shows that the forefoot is shorter than the hindfoot in all of the specimens except a very few of the smallest, and these are probably too small to be counted in the fetal period.

Another index frequently used is the ratio of the length of the extremity to the trunk length. The trunk length was not measured in these fetuses, but as a substitute for this the difference between the nose-anus length and the head length (given in the previous paper) or the spine length was used. The computed length of the fore limbs $\times 100$ divided by this spine length was determined for each 10-mm. increase in body length. The value of this index increases from 25.8 in the smallest specimens to 53.7 at 200 mm. of body length. The similar indices for the hind limb and for the same body lengths are, 17.2 and 53.0. The increase in both indices is most rapid at first, with the greatest total increase in the hind limb-trunk index.

SYMMETRY

The asymmetry of the adult human appendages has been recognized for a long time. Recognition of this condition in the fetus is of more recent date. Bartelmez and Evans ('26), Schultz ('26 and '30), and Schaeffer ('28) give ample discussion of the problem and good bibliographies. Table 2 gives the frequency and the percentage frequency of the occurrence of equal lengths or of longer right or longer left measurement. There seems to be no 'crossed symmetry,' as suggested by Schaeffer ('28), for the total length of the appendages, for

TABLE 2
Asymmetry of the eight dimensions of the two extremities

MEASUREMENT	RIGHT LONGER		LEFT LONGER		SAME LENGTH	
	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent
Fore limb	114	45.42	115	45.82	22	8.76
Arm	120	47.80	79	31.48	52	20.72
Forearm,	105	45.63	101	40.08	36	14.29
Forefoot,	88	34.92	114	45.24	50	19.84
Hind limb	112	44.62	114	45.42	25	9.96
Thigh	101	41.74	106	43.80	35	14.46
Leg	95	39.26	121	50.00	26	10.74
Hindfoot,	113	46.69	83	34.30	46	19.01

both limbs are longer on the left side, but not significantly so. We do find a 'crossed symmetry' as far as the segments of the extremities are concerned. The arm and the forearm are longer more frequently on the right side and the thigh and the leg on the left side. The left forefoot is more frequently longer (56.4 per cent of all asymmetrical cases) and the right hindfoot is longer in 57.7 per cent of the asymmetrical cases. Thus the rather marked asymmetry of the distal segment of each extremity almost seems to balance the asymmetry of the two proximal segments with a resulting very slight asymmetry of the total length of the appendages.

SUMMARY

The linear growth of the fore limb and its three parts and of the hind limb and its three parts, plotted on body length, form straight-line curves. The empirical formulae for the same eight curves plotted on body weight are all functions of the cube root of the body weight.

The sixteen formulae fit the cases fairly well, for the average deviation of the observed and the calculated values are all less than 5 per cent, except for the forearm and the leg plotted on body length. The curves plotted on body weight fit the cases better than those plotted on body length.

The lengths of the hind limb, leg, and hindfoot show a marked increase in the newborn cats over the lengths in the oldest fetuses. The other dimensions continue as smooth curves into the newborn stage.

The fore limb increases from about 20 to about 40 per cent and the hind limb increases from about 10 to a little over 40 per cent of the body length.

The hind limb and its segments increase more in length than the fore limb and the homologous parts of the fore limb. The hind limbs become heavier per unit of length, and to a somewhat lesser degree this is true of the fore limbs.

After a body weight of 10 gm. is attained, the relative lengths of the segments of each extremity change but slightly with reference to the length of the entire extremity. In the smaller specimens the percentages of both forefeet and hindfeet drop rather rapidly, while the lengths of the other parts increase, with the exception of the leg, which does not change as much as the others.

The indices of the adjacent segments of the same extremity show that the middle segment of each extremity, in general, grows more rapidly during the fetal period than the most proximal segment. The forefoot-forearm index decreases during the fetal period.

The intermembral, the humero-femoral, and the hand-foot indices all decrease, rapidly at first, and then more slowly until the newborn stage.

The two extremities do not show a significant asymmetry, but all of the segments of the extremities show a 'crossed symmetry.'

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SOME SIMPLE METHODS OF PREPARING AND
MOUNTING GROSS SECTIONS OF THE BODY,
OF ILLUSTRATING MICROSCOPIC SECTIONS,
AND OF LABELING SPECIMENS FOR
THE ANATOMICAL MUSEUM¹

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THREE FIGURES

In the creation of a museum of human morphology for teaching purposes, the writer cast about for different methods of preparing and portraying specimens. Because of the present scarcity of funds, greater reliance was placed on personal ingenuity and industry than on purchase in the growth of the collection. There was all the more reason for this because of the character of the museum, designed to parallel a course in which the different divisions of anatomy are intimately correlated. Some of the problems have been solved in a manner which yields preparations that are both instructive and esthetic, besides being simple and relatively inexpensive in execution. At the request of persons who have expressed a desire to prepare specimens in like manner, directions for the main steps of a few of the methods employed are briefly offered here.

ON THE PREPARATION AND MOUNTING OF GROSS SECTIONS

Because of legal restrictions, it is generally impossible to secure a normal adult human body soon after death for the preparation and preservation of sections in a fluid like Kaiserling's or its modifications, to maintain the natural color of

¹ Submitted for publication, January, 1933.

the parts. Instead, contrasts can be obtained by coloring them artificially—a procedure which involves more care and labor, it is true, than does the use of the Kaiserling method, but possesses distinct advantages. The selected body is thoroughly embalmed with 10 per cent formalin, then frozen and cut into sections of desired thickness with a sharp band saw in the usual way. After thawing, the sections are carefully cleaned, soaked in several changes of water, and then stored and kept moist in covered pans with neutral 5 per cent formalin until ready for mounting.

Any loose parts in the sections, such as loops of intestine, are fastened in place with gelatin. For this purpose the section, from which the preserving fluid and water have been thoroughly drained, is laid on an oiled sheet of paper and enough of the warm liquid poured into the crevices until it oozes out between the margins of the section and the paper. When it has congealed here, more of the liquid gelatin is added, sufficient to unite all loose structures firmly to one another upon its solidification.

The muscles in the section are treated with hot gelatin, colored with neutral carmine to the desired intensity. The liquid is applied with a pipette or medicine dropper, for the muscle tissue absorbs considerable quantities of it. Filling the interstices, it not only gives body to the tissue when congealed, but stains it realistically. Subsequently, the gelatin acquires a leathery firmness in the formalin in which the section is mounted.

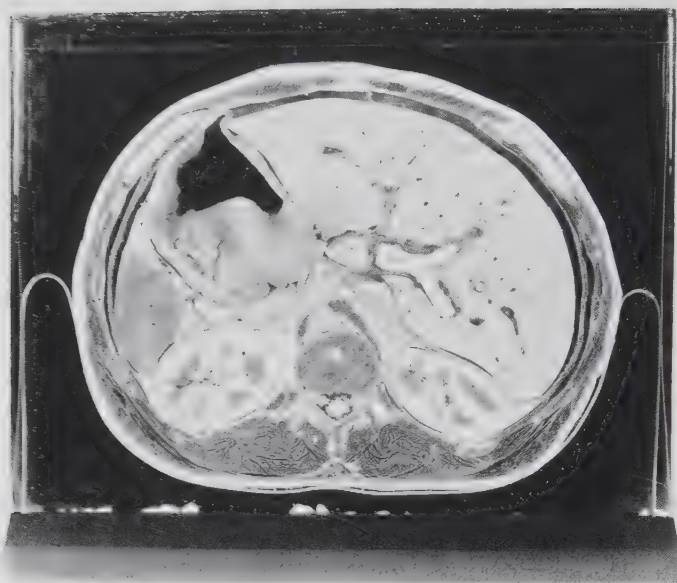
The fat in the subcutaneous areas and elsewhere in the section is demarcated by brushing on hot gelatin colored lemon-yellow with a fine suspension of lead picrate. (This substance is prepared by adding picric acid to lead acetate and washing the fine, insoluble precipitate with distilled water.) Other organs may be colored differently in a similar way, but generally there is enough contrast in appearance to make such additional steps unnecessary. It is evident that the precaution must be taken of using dyes which are insoluble in water.

Media other than gelatin, such as albumin, casein, acacia, etc., are probably quite as adaptable for this work, but the writer has not acquired sufficient familiarity with them by experiment to give definite directions for their use.

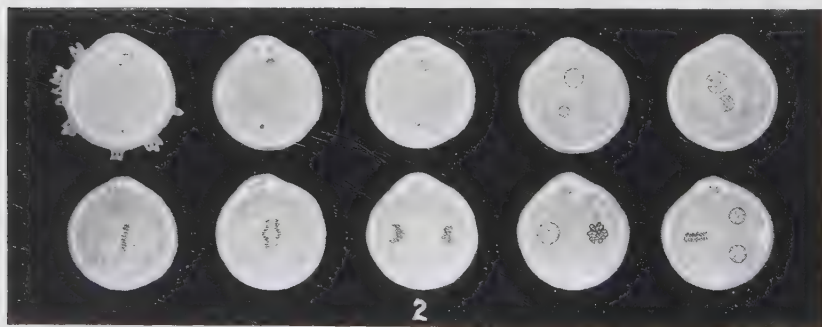
The larger vessels are filled with colored wax evenly with the surface. Preliminary to this, all coagula of blood are removed and the depth of a vessel's lumen stuffed with cotton. The remainder of the lumen receives the molten wax (one part of paraffin, two parts of pure white beeswax, and the proper amount of artist's oil color), which is allowed to drop into the cavity from the tip of a hot iron to which the colored wax plate is held. The cotton plug becoming saturated with the wax serves to hold the colored mass in place after it has hardened. Vermilion is indicated conventionally for the arteries, and cobalt blue for the veins. A sufficient quantity of the oil paint must be used to produce a color of considerable intensity, for the light blue wax, with which the writer at first filled the veins, shows after the lapse of a year a noticeable amount of fading.

Smaller vessels, including the larger lymph channels, such as the thoracic duct, may be demarcated by glass beads of proper size and color fastened into their lumina. The various ducts, such as bile duct, ureter, ductus deferens, etc., are labeled in a similar way.

For mounting the section in the museum jar, instead of suspending the section which would cause its distortion, or of preparing a rack of glass rods which is difficult to make, the writer employs strips of the non-corrosive monel metal. The strip, bent to conform to the outlines of the lower half of the section, and its ends serving as legs which are adapted to fit snugly against the inner sides of the jar, forms a supporting hammock for the preparation (fig. 1). The strip used, being 1.5 mm. thick and 2.2 cm. wide, is firm enough for support and at the same time sufficiently pliable to permit its bending into the desired curves. The section itself should be tied tightly to the strip by a few loops of silk thread, to prevent any bouyancy it may possess because of air bubbles and the



1



2



3

Figures 1 to 3

resulting separation from the strip when the jar is filled with the preserving fluid.

The sections are mounted in a 5 per cent solution of chemically pure, neutral formalin. Traces of acid have a deleterious effect on the colors, and in time cause a disintegration of the gelatin. For this reason, washed white marble chips are scattered on the floor of the jar, which will neutralize any acid that is set free by the preparation or by the deterioration of the formalin.

The transverse sections of the body prepared by the writer were cut at an approximate thickness of 1 inch, and were mounted in broad flat museum jars of corresponding front-to-back width in order to bring the viewed surfaces of the sections against the glass. The inside dimensions of the jars for the sections of the trunk are: front-to-back width, 3.5 cm.; breadth, 37 cm.; and depth, 31 cm. The sections of the head, neck, and extremities were mounted in correspondingly smaller jars. It is clear that the aquaria-like containers made by hand, and used in several anatomical laboratories, are quite as suitable for the mounting of such sections.

Because most museum specimens are set off best against a black background, the back of the jar and the sealed margins of the cover are painted with black enamel. This is more effective and costs much less than the plates of black glass which many preparateurs place in the jars instead. Finally, the narrowness of the jars demands that they be provided with a wooden base for support (fig. 1).

ON THE ILLUSTRATION OF CYTOLOGIC AND HISTOLOGIC SECTIONS

Besides the preparation of stained celloidin sections through entire organs, such as lungs, heart, spleen, kidneys, uterus, prostate, etc., mounted in balsam between panes of glass, the writer has devised a method of illustration which has wide possibilities in the portrayal of microscopic specimens for the museum.

The method can best be indicated briefly by referring to a specific series, for example, the stages of fertilization, matura-

tion, and division of the ovum. The magnified outline of a section of the cell is cut from a sheet of delicately tinted wax of desired thickness (some 2 mm.). From this plate representing the cytoplasm, a circular area as large as the image of the nucleus is cut—if the picture is to show a stage with nuclear membrane intact—and a disk of white wax of corresponding size inserted and fused in place. Here, on one surface the nuclear structure is painted with oil colors. Similarly, granules may be stippled and mitochondria shown on the cytoplasmic field. The appearance of globules and vacuoles may be produced by forming concavities of variable size on the same surface with the rounded ends of hot glass rods or test tubes and absorbing the melted wax. For mitotic figures, the chromosomes are painted in their proper places, and spindle fibers and astral rays engraved with pen and India ink; for as the pen cuts a fine groove into the wax, the ink flows to fill it. The zona pellucida may be shown as a ring of more deeply colored wax encircling the cytoplasm.

Such preparations are now fastened in proper sequence on a large pane of glass with the painted side up. When this side is held toward the light, so that the opposite side can be viewed, the light shimmering through the translucent wax softens the lines and gives a sense of body and protoplasmic effect, which a colored sketch on paper is unable to give. The effect is enhanced by blackening the surrounding glass with enamel (fig. 2). To do this it is essential that the margins of the wax picture are tightly sealed to the glass, for the minutest pore will cause the fluid enamel to seep between. Finally, the individual preparations are sprayed with shellac, and the entire group is framed to be placed in front of a window or a shadow box. The length of the frame pictured in figure 2 is 86 cm. (34 inches) long and 34.5 cm. (13.5 inches) wide.

It is obvious that any other microscopic preparation can be portrayed for the museum quite as effectively by the method indicated.

ON THE LABELING OF SPECIMENS

The simplest label base is a block of pine wood, in cross section a right isosceles triangle, as shown in figure 3. The size of the block will vary according to the amount of descriptive matter it is designed to carry. The largest block used by the writer measures 4.2 cm. for its equal sides, 6 cm. for its hypotenuse, and 20.3 cm. for its length, while the smallest has dimensions, respectively, as follows: 3.2, 4.5, and 4 cm.

The ends and planed surfaces of the blocks are smoothed with sandpaper and covered with black enamel, except the hypotenusal surface. On this the label of black glossy paper with white lettering is glued firmly. The effect is the same as if the lettering had been done on the block itself. To prepare the label, the original lettering is made with India ink on white cardboard, while the reversal of white and black is achieved photographically.

The finished blocks are simple and neat, lie securely, and are effective whether placed on top of the jars or in front of the preparations; they do not detract from the specimens, yet display plainly the information they carry.

EMBRYOLOGICAL PATTERN PERSISTING IN THE ARTERIES OF THE ARM

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THREE FIGURES

In a recent dissection, an interesting condition was disclosed in which indications are clearly retained of nearly all the stages through which the arteries of the arm have passed in their course of development. The specimen contributes to the viewpoint that anomalous connections between the stems of two great arteries do not develop haphazardly, but that such connections may occur as a manifestation of some more primitive conditions.

Figures 1 and 2 are drawings of the arterial distribution revealed in this specimen; figure 2 showing the topographic position of the artery to the adjacent structures in the arm and forearm, and figure 1 showing the complete course of the artery and its branches to their digital termini. The explanation of this anomaly can be found directly in the developmental stages of these vessels. In reviewing their embryonic origin, we can recognize five stages (Senior, '26). Figure 3 is a chart giving diagrammatically the characteristics of these five stages of development of the brachial artery, with a similar diagrammatic interpretation of the present specimen.

Stage I. Originally the a. subclavia extends to the wrist, where it terminates by dividing into terminal branches for the fingers. The distal portion of this artery becomes the a. interossea of the adult.

Stage II. The a. mediana arises from the a. interossea and becomes larger, the a. interossea subsequently undergoes re-

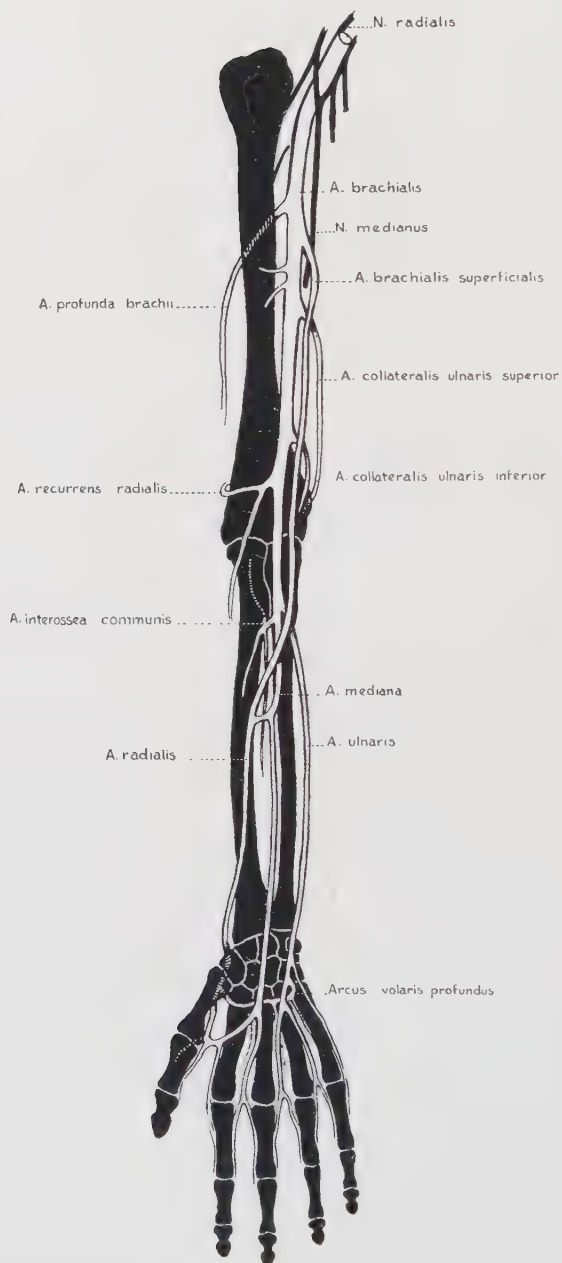


Fig.1 Schematic picture of arteries of right arm, showing the retention of developmental conditions.

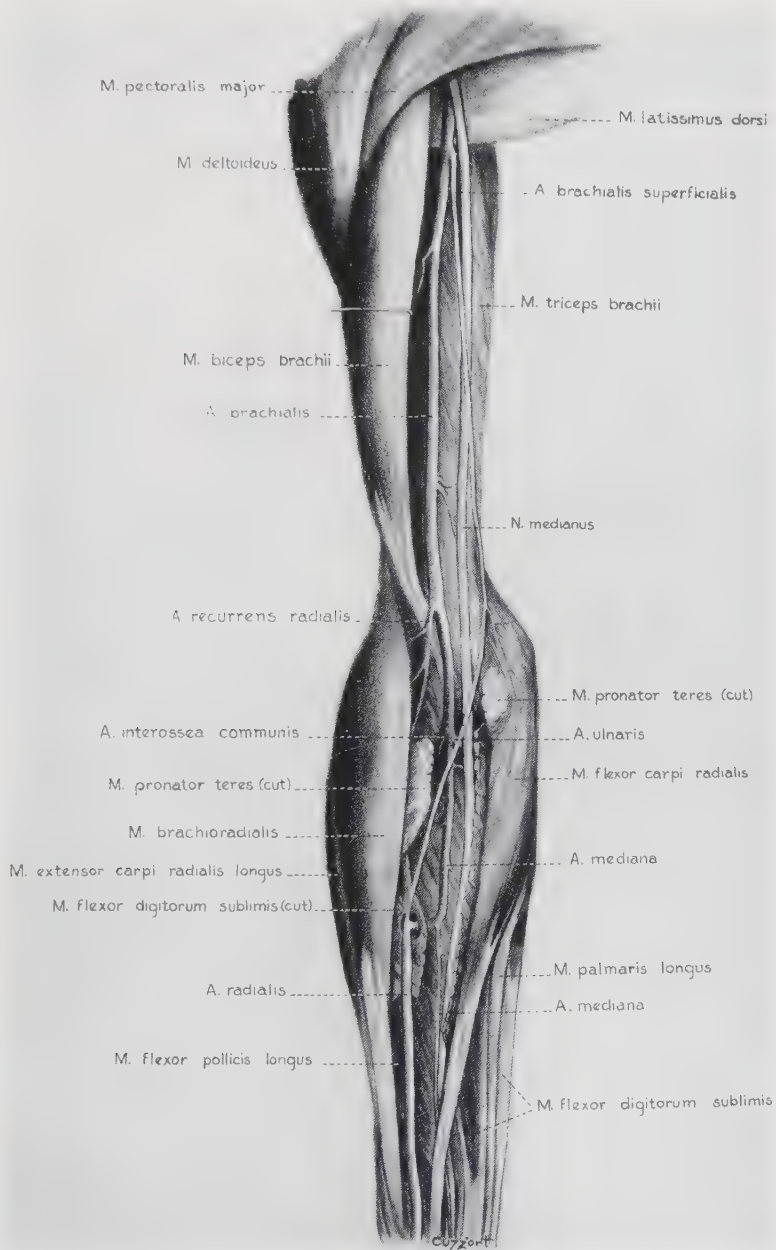


Fig. 2 Variation of arteries of right arm, showing a. brachialis superficialis and a. mediana and other arteries in their topographical position.

troggression. During this process the a. mediana fuses with the lower portion of the a. interossea and ultimately forms the main channel for the digital branches becoming the principal artery of the forearm.

Stage III. In embryos of about 18 mm. the a. ulnaris arises from the a. brachialis and unites distally with the a. mediana to form the superficial carpal arch. Digital branches arise from this arch.

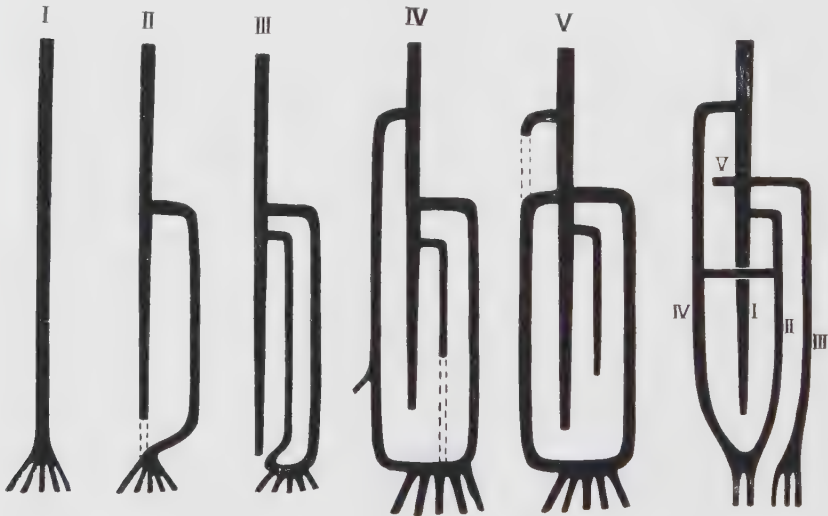


Fig. 3 Diagrams illustrating the development of the arteries of the arm (after Piersol) and a similar diagram of the present anomaly.

Stage IV. In embryos 21 mm. long the a. brachialis superficialis develops in the axillary region and traverses the medial surface of the arm and runs diagonally from the ulnar to the radial side of the forearm to the posterior surface of the wrist. There it divides over the carpus into branches for the dorsum of the thumb and index finger.

Stage V. Finally three changes occur. When the embryo reaches the length of about 23 mm., the a. mediana undergoes retrogression, becoming a small, slender structure now termed the a. comes nervi mediani. The a. brachialis superficialis gives off a distal branch which anastomoses with the arcus

volares superficialis already present. At the elbow, an anastomotic branch between the a. brachialis and the a. brachialis superficialis becomes enlarged sufficiently to form with the distal position of the latter, the a. radialis as a major artery of the forearm; the proximal portion of the a. brachialis superficialis atrophies correspondingly.

Comparison of the condition shown in this specimen with these developmental periods reveals an obvious advance beyond the first stage. The second stage is represented by the unusual thickness of the median artery to its anastomosis with the radial, and with the retention of palmar branches to the second and third fingers.

Evidence of the third stage is seen in the union of the three ulnar aa. volares communes with the ulnar artery as an inhibited development of the volar arch. The development of the fourth stage is entirely retained, with the result that the final outcome differs with a normal fifth stage in the following points.

a. The a. mediana keeps its contact with the second digitalis communis.

b. No anastomosis of the a. radialis with the palmar arch is present.

c. There is no r. dorsalis of the a. radialis.

d. The anastomosis between the lower part of the a. brachialis and the a. brachialis superficialis does not develop, although we have a branch of the a. recurrens radialis, at the site of that anastomosis.

We can see from the above comparison that the anomaly clearly represents an inhibited development of the vessels at different stages of the embryonic period.

The literature (Breme, 1899; Dubreuil-Chambardel, '06; Schwalbe, 1898) shows that a retained a. brachialis superficialis is usually associated with a retarded development of the volar arch. The present specimen does not give any clue as to whether one or the other might be regarded as the primary factor in such disturbed growth. The case shows that one should not be content to explain arterial anomalies

with unusual anastomoses in the light of a purely fortuitous development.

DETAILED DESCRIPTION OF THE SPECIMEN

One inch below the origin of the *a. profunda brachii* arises the *a. brachialis superficialis*. The latter runs anterior to the *n. medianus* as far as the elbow, where it gives off a little branch which supplies the *m. flexor carpi radialis*. This small branch, the *a. plicae cubiti superficialis*, arises usually from the *brachialis*. After giving off this branch, the superficial artery turns toward the radial side, crossing the *n. medianus* and the *m. pronator teres*, and anastomoses with the *a. mediana* 5 inches above the wrist.

The *a. recurrens radialis* arises from the *a. brachialis* at the normal site of the *a. radialis*, and has a muscle branch suggesting the course of the latter.

In this specimen, the *a. mediana* originates at the elbow as the continuation of the *a. brachialis* extending as the major trunk ($\frac{1}{4}$ inch in diameter) to about the middle of the forearm, between the *mm. flexor digitorum sublimis* and *profundus*. At the origin of the *a. mediana*, the *a. brachialis* gives off two other branches, the *a. ulnaris* going to the ulnar side of the elbow, and the *a. interossea communis*, to the radial side. There it makes an abrupt lateral turn, piercing the *m. flexor digitorum sublimis*, to be continued downward as the *a. radialis*. At this point it is joined by the *a. brachialis superficialis* as an anastomosis.

The *a. mediana* is separated from the *a. interossea communis* by an anomalous head of the *m. flexor pollicis longus* which arises from the medial epicondyle below the *m. pronator teres*. From the point of its lateral bend the *a. mediana* becomes very much thinner. It follows the course of the *n. medianus* under the *lig. carpi volare* and becomes the second *a. digitalis volaris communis*, also giving off the *a. volaris indicis ulnaris*, *a. volaris digiti III radialis*, and an anastomotic branch to the *a. radialis*. The *a. radialis* resulting from the anastomosis described above, is of normal size and posi-

tion. It runs superficially to the *m. flexor digitorum sublimis* and between the *mm. brachio-radialis* and *flexor carpi radialis* following the normal course of the artery.

On the dorsum of the hand, the *a. radialis* divides into two terminal branches, the *arcus volaris profundus* and the *a. princeps pollicis*. The former gives off three *aa. metacarpeae volares* between the second, third, and fourth intermetacarpal spaces. The *arcus volaris profundus* gives origin to the *aa. metacarpeae dorsales*. They pierce the *interossei* muscles to reach the dorsal side.

A *ramus carpeus dorsalis* of the *a. radialis* is entirely missing. The *a. princeps pollicis* has a branch that runs volarly over the *m. adductor pollicis* and divides into the *a. volaris indicis radialis* and into an anastomotic branch to the *a. mediana*.

The *a. ulnaris* gives off in the hand, the two *aa. ulnares digitales volares communes*, the *a. digiti quinti ulnaris* and also an anastomotic branch to the *arcus volaris profundus*.

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HUMAN BRACHIAL PLEXUS UNITED INTO A SINGLE CORD

DESCRIPTION AND INTERPRETATION

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FOUR FIGURES

Evidence has shown that anomalous conditions in the peripheral nervous system frequently result from some change in the vascular system whereby the latter imposes itself in the path of the former very much in the same way as the experimentalist interferes with the normal course of development (Michelson, Müller, and Ruge).

The case under consideration reveals an anomaly of the axillary artery associated with an irregularity of the brachial plexus. Two similar cases of a brachial plexus with a single cord have been reported by Müller ('05) and by Michelson ('20). They were principally interested in the peculiarities of the axillary artery, however, for their description of the brachial plexus is only sufficient to recognize the resemblance between the cases reported by them and the present one. For this reason, detailed comparison of the plexus is not available, and they did not discuss the association of these anomalies. The present case is reported to consider this latter point.

Figure 1 is an illustration of the dissection showing the single cord of the brachial plexus and the accompanying arteria axillaris. Although the condition presented by the brachial plexus comprises a very gross anomaly in the union of all its cords and divisions, careful detailed dissection with the aid of a dissecting microscope after proper maceration (method proposed by Kerr, '18) failed to disclose any ab-

normal differences in the origins, courses, or destination of its individual nerve elements (fig. 3). Hence the abnormality of the plexus lies only in its extraordinary form. The anomaly has been found to have had no bearing upon the function of the arm, as confirmed by the hospital report.

The axillary artery lies medially to a single united cord, instead of being located between the three normal cords of

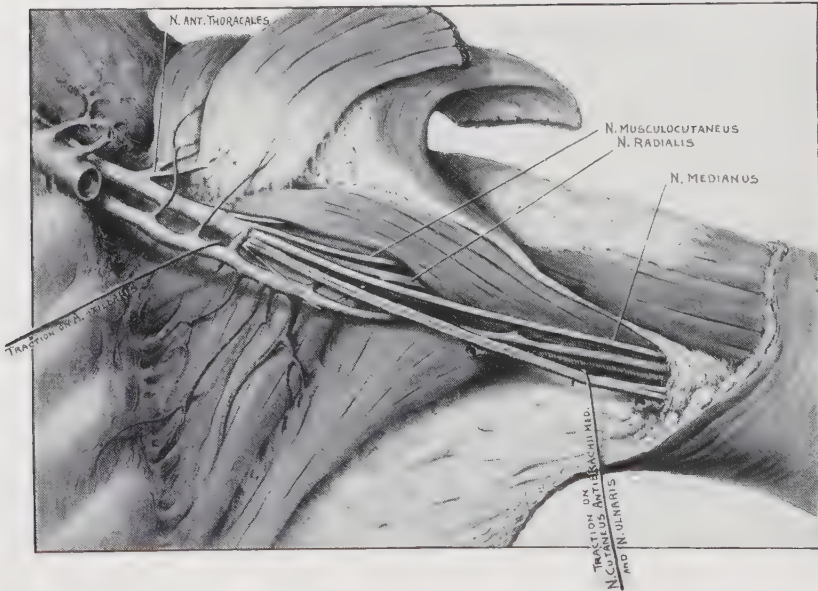


Fig. 1 United cords of the brachial plexus lying lateral to the axillary artery. A bundle encircles the axillary artery to form a root of the radial nerve. The axillary vein is cut, the stumps are visible medial to the artery.

the brachial plexus in a manner which gives them their usual designation, medial, lateral, and posterior cords. In the middle of the arm the artery divides into two branches of equal size. One branch, lying anteriorly to the n. medianus, assumes the position of the a. radialis in the distal part of the arm. The other branch corresponds to the a. ulnaris (lying posteriorly to the n. medianus) and gives origin to all the branches that normally arise from the a. interossea.

The united cord revealed a hole in its lower end, transmitting a small blood vessel (fig. 2) which, according to its position, appears to be the embryonic *a. axillaris profunda*. We call attention to this opening because it lies between the two heads of the *n. medianus*.

Emerging from the interval between the anterior and middle scalene muscles, the nerve roots C5, C6, C7, C8, and T1 entered into the formation of this single-corded brachial plexus in the following manner:

The fifth and sixth nerves (each about 4 cm. in length) unite to give rise to a common trunk (2.5 cm. long), which then joined the nerve C7.

The seventh nerve, before joining the common trunk, formed the middle trunk ($4\frac{1}{2}$ cm. long). At its proximal end it gave off a few fibers to the eighth nerve. The eighth nerve (2 cm. long) joined the T1 (2 cm. long) forming the common trunk ($4\frac{1}{2}$ cm. long). The inferior trunk then joined the united cord which gave off the terminal branches.

In explanation of this anomaly, Müller and Senior¹ have demonstrated that the nerve plate of the embryo becomes perforated by a number of vessels (fig. 4). Of these perforating vessels two become permanent, namely, the *a. transversa colli* and the *a. axillaris profunda*, the latter becoming the *a. axillaris* of the adult. While Müller believes that the perforating vessels are all arteries, Senior is inclined to think, and we are of the same opinion, that only a few are arteries, and the network that Müller shows in his pictures represents mostly veins. These perforations for the passage of vessels develop into the loop for the *n. medianus* and the *n. thoracales anteriores*. Further development of the nerve plate consists in its splitting into bundles and in an enlargement of the openings through which the arteries pass. In the present case, the *a. axillaris profunda* did not develop in the normal manner, remaining in a rudimentary state. It pierced the nerve plate originally, but its subsequent development was arrested so that further separation of the plate did not ensue.

¹ Personal communication from Doctor Senior.

Thus the foramen remained small as in the embryo. Study of the network of the plexus after maceration shows that the nerve bundles were differentiated from a normal anlage, but kept their embryonic juxtaposition through failure of cleavage which normally is induced by growth of the profunda artery.

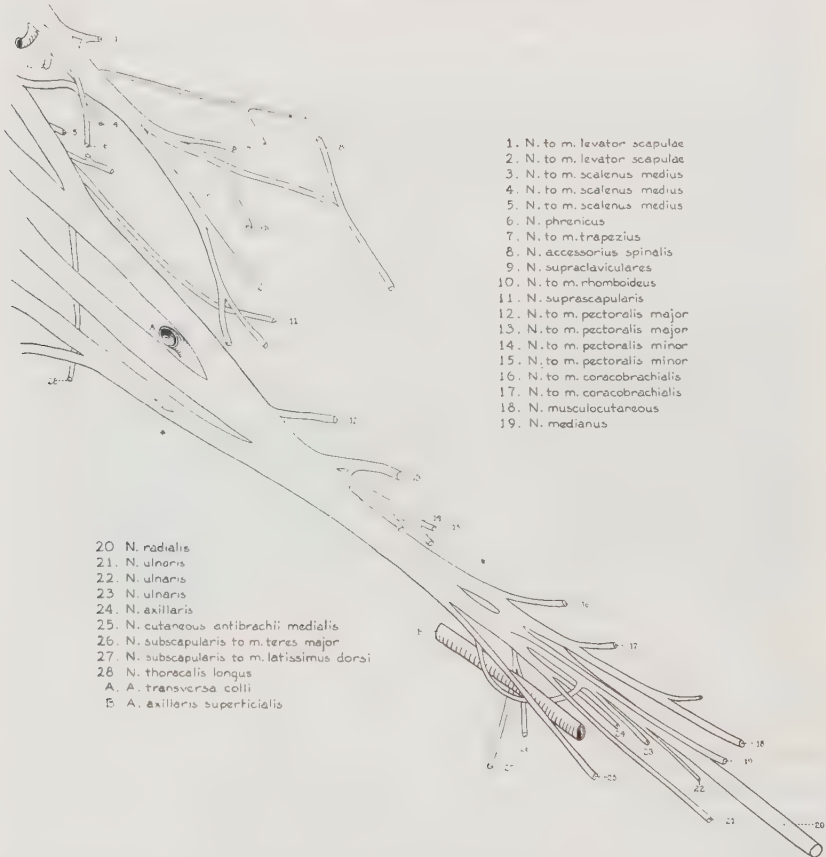


Fig. 2 The brachial plexus as it appeared after cutting it out of the body.
 *, the hole in the united cord.

The indications are that the usual separation of the cords from each other is due to a normal development of the a. axillaris profunda of the embryo, which persists in the mature individual as the a. axillaris.

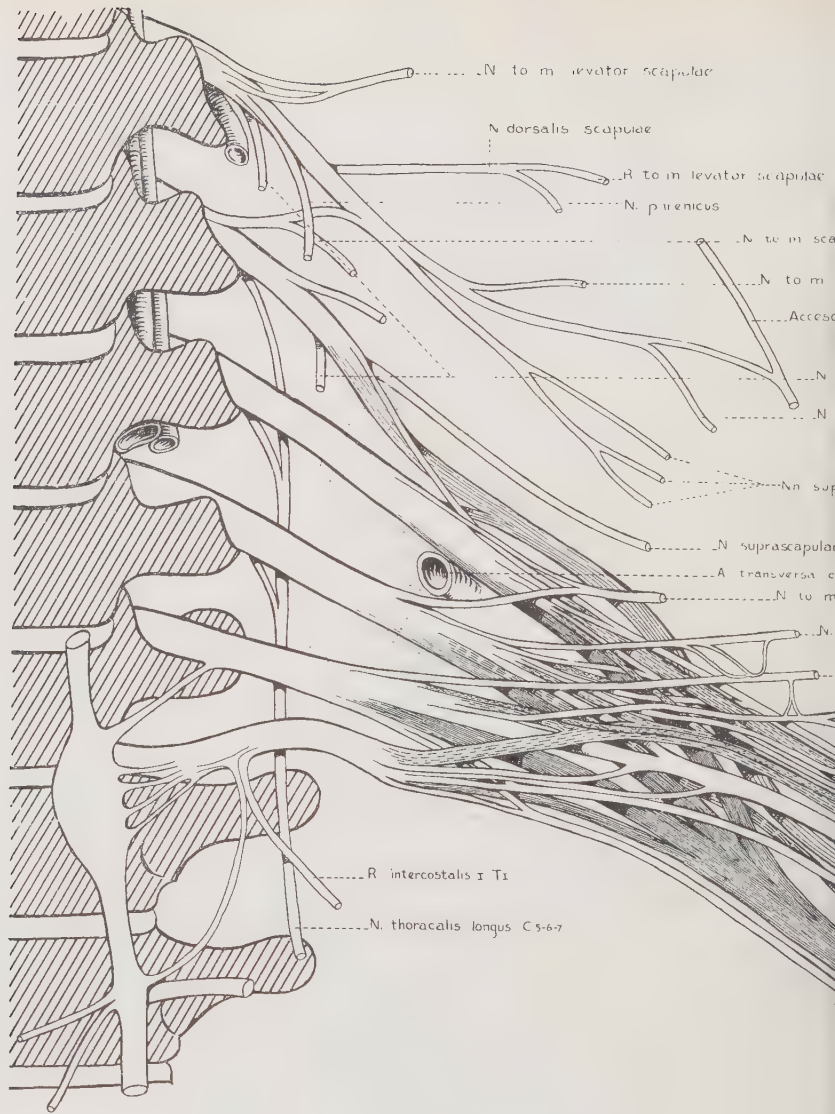


Fig. 3 The brachial plexus

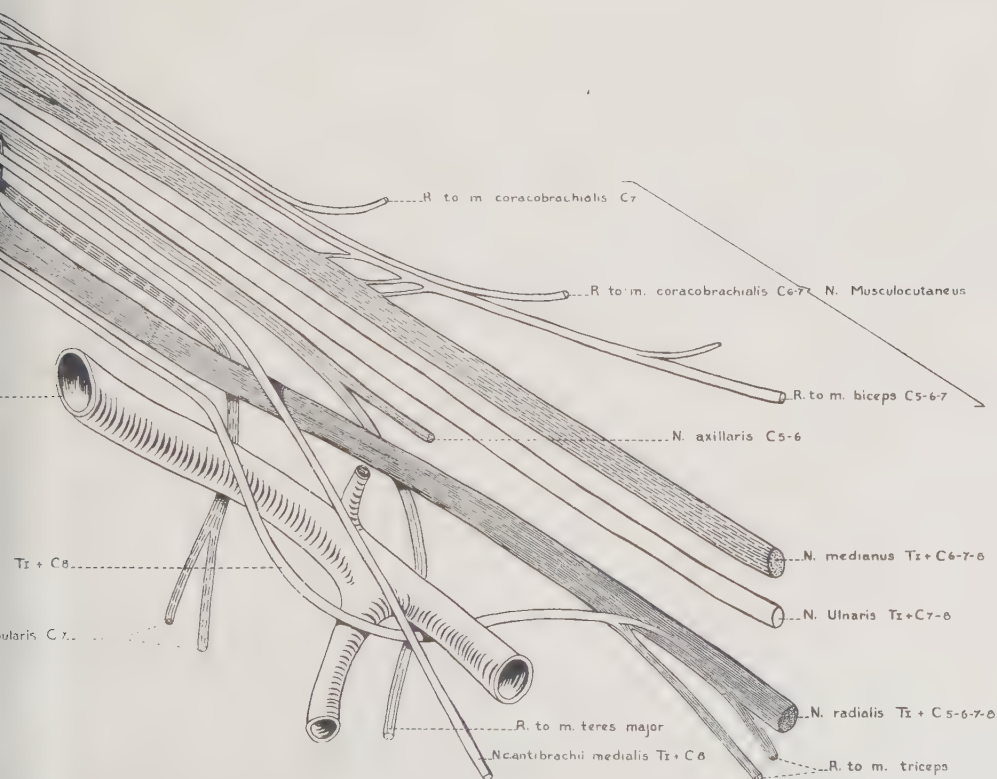
C.4
alis

enus medius
mboideus C.4

res C.4.5

s major C6-7
oralis major piercing pectoralis minor C7
ectoralis minor C7-8
o m. pectoralis minor C8
o m. pectoralis major C8

Nn thoracales anteriores



the components revealed by tracing their fibers.

The solid constitution of the cord as observed in this case should be regarded as a result of non-development, or retarded development, of the foetal a. axillaris profunda. Under these conditions, the existing a. axillaris is a compensatory over-development of the a. axillaris superficialis.

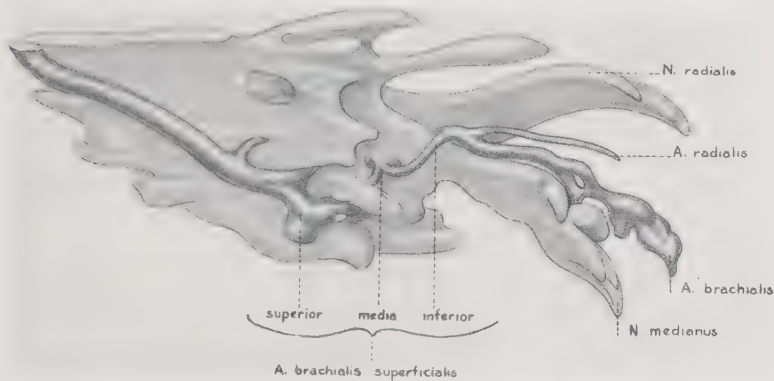


Fig. 4 Brachial nerve plate of an embryo (after E. Müller).

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FORTY-NINTH ANNUAL SESSION

University of Cincinnati College of Medicine, Cincinnati, Ohio

April 13 to 15, 1933

ABSTRACTS

PAPERS TO BE PRESENTED

(The numbers in parenthesis indicate the order in which the papers appear on the program of the meeting; the numbers in italics give the serial number of the abstract as printed herewith.)

- (60) 1. *Some studies on the reaction between living cells and chemical substances in the 'moat' chamber introduced into the rabbit's ear.* R. G. Abell and E. R. Clark, Department of Anatomy, University of Pennsylvania.

Following the injection of phosphate buffers at both pH 6.2 and 7.4 into moat chambers, a recession of the circulation away from the source of the buffer was observed. In the case of the buffer at pH 6.2, the extent of recession was 1.086 mm., while following the injection of the buffer at pH 7.4 the circulation retracted 0.63 mm. This recession was due chiefly to the destruction of the tips of those vessels closest to the source of the phosphate buffer. A precipitate was formed in the region just in front of the most advanced vessels. No precipitate was formed farther away from the vessels than approximately 0.125

mm., and none was formed proximal to the last capillary. Part of the precipitate disappeared within a few weeks, but a part was permanent, particularly that formed with the buffer at pH 7.4.

Following the injection of methylene blue into the moat, many cells in the region just in front of the most advanced capillaries stained a brilliant blue. The region of staining coincided almost exactly with the region of formation of precipitate following the injection of phosphate buffers. Following the passage of oxygen through the moat, many structures stained blue in the entire region between the moat and the vessels, a distance of approximately 1 mm.

- (21) *2. Nutritional inhibitors of hormonal action.* S. B. D. Aberle, Department of Obstetrics and Gynecology, Yale University School of Medicine.

The following experiment was undertaken to determine whether or not a hormone could produce its characteristic effect upon cells altered in their morphological characteristics by a defective diet.

Rats on an A-deficient diet and controls on an adequate diet were ovariectomized. All of the animals were kept until the vaginal cells in the A-deficient animals had been cornified for at least a week. The substance extracted from human placentae had been standardized for the hormone which produces vaginal cells similar to those seen in pregnancy. Each dose of hormone was administered in 3 or 6 injections given at 6-hour intervals.

Seventeen doses of hormone of various strengths were given to the A-deficient rats and seven doses of hormone to control rats. In some instances the condition of the vagina was followed by smears. All the rats were killed at the time the vagina of the controls showed an effective response to the hormone. In none of the smears or histological sections of the vagina of the A-deficient rats were there found any cells resembling those of pregnancy. The epithelial cells showed a low cornification, typical of avitaminosis of A. This was in contrast to the controls, which showed in each instance the pregnancy cell in the mucosae.

Conclusion: In certain states of vitamin A deprivation in rats, a hormone is incapable of action.

- (33) *3. Pigmentation of meninges in Norway rats.* W. H. F. Addison and Doris A. Fraser, Department of Anatomy, University of Pennsylvania.

Melanotic pigment is constantly present in the cranial meninges of the Norway rat. In adult animals the pigment is readily visible when the brain is exposed. It is not evenly distributed, but is more abundant in definite areas. On the dorsal aspect of the brain the pigment is along the dural vessels between the cerebellum and cerebrum, and in the membranes behind the olfactory bulbs. It often forms a conspicuous area in the pia along the rhinal fissure. Ventrally it is seen throughout the base of the skull ensheathing blood vessels and nerves. The melanophores have a small cell body with long branching processes. The pigment granules are abundant throughout the body and processes.

In the newborn the pigment is not seen grossly. However, when shreds of the meninges are examined microscopically, the melanotic cells are seen in certain areas. These cells are scattered diffusely and show no evidence of orientation around blood vessels as in the adult. The position of the nucleus is much more

evident than in the adult because of the sparser distribution of the granules overlying it. Similar pigment cells have been reported in a small number of other mammals, and are very abundant in lower vertebrates. (See demonstration no. 4.)

- (20) 4. *Endocrine components of the human ovary.* Edgar Allen, University of Missouri, and J. P. Pratt, Henry Ford Hospital.

Ovarian tissues removed at operation at various stages of the menstrual cycle have been studied with the object of more accurately determining the condition of follicles and corpora lutea responsible for ovarian endocrine activity. Cumuli containing ova were cut from the walls of the larger follicles and sectioned for examination and diagnosis of normality or atresia. Sections of selected regions of cortex were cut for study of primary follicles and possible proliferative activity of the germinal epithelium.

Study of ninety follicles ranging from 3 to 10 or more millimeters in diameter has revealed no ova in maturation divisions. Several specimens were found with cumuli containing lakes of secondary liquor folliculi and various stages of corona formation. In some of these the structure of the outer layer of the granulosa cells showed signs of activity, possibly secretory in nature.

From the above study it is hoped to reach a generalization as to the number, size, and endocrine function of the larger follicles at various stages of the menstrual cycle. A great majority are atretic. It is clear that in the human ovary there is radical selective elimination of follicles relatively late in their development.

Careful search of the germinal epithelium of several ovaries shows continued proliferative activity in the formation of new ova.

- (18) 5. *Endogenous stimulation of albino rat fetuses.* A. W. Angulo y González, The Wistar Institute of Anatomy and Biology.

Albino rat fetuses of about 16 days, with intact circulation, that could not be aroused by tactile stimulation responded spontaneously with lateral flexion of the head, or with lateral flexion of the trunk with fore limb movement, when the umbilical cord was ligated.

Fetuses of about 18 days, with intact circulation, that could not be aroused by tactile stimulation of the hand, responded spontaneously with waving of the hand shortly after ligation of the umbilical cord. These discrete movements were followed by total mass movements.

Fetuses of about 19 days, with intact circulation, that could not be aroused by tactile stimulation of the hind leg, responded with movement of the hind leg, followed by discrete movements of the hand and finally by total mass movements, when the umbilical cord was ligated.

If the umbilical cord of any fetus is ligated, it will recapitulate the maximum degree of physiological activity it has achieved, but in the inverse order of its normal development.

The foregoing conclusions can be summarized into the following law: Endogenous stimulations affect directly the motor centers, and act first on the most recently developed units. This is due to the fact that the new growing

mechanisms have the highest physiologic gradient and are, therefore, affected first by the relative increase of metabolites or any stimulating substance in the blood.

- (19) 6. *The fissula ante fenestram in an adult human ear.* Barry J. Anson and J. Gordon Wilson, Departments of Anatomy and Otolaryngology, Northwestern University Medical School.

It has long been known that there exists within the lateral wall of the osseous vestibule a minute fissure which extends from the tympanic cavity inward through the bone to a point just in front of the vestibular window.

Models of this channel show that in the adult ear it begins as a crevice-like continuation of the vertical sulcus which, on the labyrinthic wall of the tympanic cavity, marks the posterior extent of the promontory. After traversing the bone in an inferior and posterior direction the inner orifice of the fissula becomes continuous with the cavity of the internal ear just medial to the anterior extremity of the vestibular window, at a point where the vestibule becomes the scala vestibuli.

In its course the long axis of the fissula changes through about 90°, being at first directed inward, then backward.

Models representing the cleft-like space of the fissula show that its anterior and posterior margins are concave backward; also, that its space communicates at several points with haversian canal-system of the surrounding bone, through which communications the connective tissue of the fissula receives small blood vessels.

Near the extremities the tissue enclosing the fissula is largely bone; otherwise, the fissula rests in modified cartilage (interglobular spaces).

- (78) 7. *The form and relations of the crypt systems in the postnatal palatine tonsil.* L. B. Arey, J. T. Milton, and W. L. Minear, Northwestern University Medical School.

Reconstructions of human tonsils at 3, 21, and 65 years show about thirteen crypts at each stage. The larger ones are located superiorly, while a decrease in size and complexity occurs inferiorly. An especially large crypt opens centrally on the superior third. In size this equals the remaining crypt systems combined. Crypts vary from simple pockets to highly branched, antler-like systems. Especially in such complexity characteristic of the largest system just mentioned. In this instance fifth-order branching occurs. There may be anastomoses between branches of the same crypt.

At 3 years the fundamental plan of the mature tonsil is laid down, but the final complexity is lacking. The 21-year stage apparently represents structural maturity. At 65 years there is marked epithelial atrophy. The method of this process is illustrated by thirteen cystic portions of crypts; some of these show slight epithelial continuity with the mother crypt, while others are detached and further advanced on the degenerative course.

Mucous glands lie along the posterior tonsillar border. About twelve ducts are closely associated with the tonsil. At 3 years none definitely emptied into

a crypt; at 21 years there was one such connection; at 65 years six ducts drained into crypts. Nodules of cartilage occur at the periphery of the 3- and 21-year stages.

- (25) 8. *Mammary development in the rabbit.* S. A. Asdell, J. Hammond, and G. W. Salisbury, Cornell University, Ithaca, and Cambridge University, England.

Recent work by Corner, Turner, and others has shown that injection of oestrin produces duct growth in the mammary gland of the rabbit, addition of progestin carries development further, but an anterior pituitary extract is needed to give complete growth. Attempts are being made to work out the mechanism by which the glands are normally developed. Ovariectomy during pseudo-pregnancy arrests development. It is promoted when corpora lutea are present, whether induced by sterile coitus, anterior pituitary extracts, or by pregnant human urine. The influence is therefore not one of coitus on the anterior pituitary, but of corpus luteum either directly on the mammary gland or through the pituitary. Prolongation of the life of the corpus luteum by Loeb's hysterectomy technique maintains the mammary gland at the pseudo-pregnant level, but does not carry growth further, although the corpora lutea are effective as suppressors of ovulation for 24 days instead of the usual 16. Exposure of the rabbits to a succession of pseudo-pregnancies by sterile copulation or by anterior pituitary extracts does not carry mammary development further than is achieved in a single pseudo-pregnant period. Development during pseudo-pregnancy is therefore due to a corpus luteum influence. As conditions in the first half of pregnancy are similar, probably the corpus luteum influences mammary development during this time, but not during the second half.

- (51) 9. *Cortin treatment after hypophysectomy in the rat.* Wayne J. Atwell, Department of Anatomy, University of Buffalo.

Since hypophysectomy is followed by atrophy—and presumably lowered function—of several other endocrine glands, it may be assumed that the syndrome characteristic of hypophysectomy includes the effects resulting from reduced function (if any) of these glands. Other investigators have shown this to be true for the thyroid and the gonads. The availability of a potent extract of the adrenal cortex (Cortin, Hartman) has made it possible to test the role of another important organ.

Hypophysectomized rats were injected subcutaneously twice daily with Cortin in doses of 0.5 cc. (first series) and 2.0 cc. (second series). This treatment partially relieved the characteristic asthenia and hypothermia and partially restored spontaneous activity. It failed to repair the atrophy of the gonads, the thyroid or the adrenal cortex. The interrupted oestrous cycles of the females were not restored. There was no restoration of the growth rate. This is a continuation of work previously reported.

- (36) 10. *The frequency of atypical neurones in the spinal ganglia under normal conditions and after lesions of the roots, nerve, or ganglion.* R. W. Barris, Institute of Neurology, Northwestern University Medical School. (Introduced by S. W. Ranson.)

In the normal spinal ganglia of cats the vast majority of the cells belong to the simple unipolar type. Of the atypical cells the most numerous are those associated with end bulbs, which represent the terminal expansions of processes arising either from the cell body or from the axon. Cells with pericellular or periglomerular plexuses are much less numerous and other atypical forms are very rare. In a normal ganglion containing 31,000 cells there never were found more than thirty-nine atypical cells. The smallest number found in any ganglion was seventeen.

In four cats the left sciatic was sectioned at the middle of the thigh; in nine others the lumbosacral dorsal roots were cut close to the cord; and in another five cats the seventh lumbar and first sacral spinal ganglia were incised with a scalpel. After survival periods, varying from 3 to 108 days, the affected ganglia were removed along with the control ganglia of the opposite side and prepared by the pyridine silver technique. Section of the sensory fibers in the peripheral nerve or dorsal roots at considerable distances from the ganglion does not cause an increase in the number of the atypical cells, but direct incision of the ganglion produces an increase in the number of pericellular and periglomerular plexuses.

- (40) 11. *The trophic influence of the spermatic nerve on the testicle.* Donald H. Barron, Department of Anatomy, Union University, Albany Medical College. (Introduced by W. M. Baldwin.)

The trophic influence of the external spermatic nerve upon the testicle has been investigated in a series of rats. Faradization of the nerve in the abdomen is followed by a decrease in the temperature of the testicle of between 0.5°C. and 1°C. Section of the nerve in the abdomen is followed by a corresponding increase in the temperature of the testicle. Hence it is concluded that vaso-constrictor fibers reach the testicle through this nerve.

After section of the external spermatic nerve, the testicle undergoes degeneration similar to that produced by heat, x-ray, or inflammation. Though the temperature increase following section of the external spermatic nerve has never been observed to continue for more than 20 days, the degenerative processes continue much longer. This degeneration appears to be due to the hyperemia resulting from vaso-dilation rather than to the increase in temperature that results from section of the external spermatic nerve.

- (3) 12. *The structural elements of the synapse.* G. W. Bartelmez and N. L. Hoer, Department of Anatomy, University of Chicago.

Large myelinated fibers from sacculus envelop lateral dendrite of Mauthner's cell in *Ameiurus* and terminate on its surface; a mechanism subserving mass reactions. Root fibers and Mauthner's fiber are well myelinated in 10-mm. fish. In longitudinal section the former terminate abruptly in end bulbs; these reach a diameter of 7.2 μ . Myelin sheaths extend up to end bulbs. The club endings

are as difficult to preserve as the typical 'end feet' of Held which cover cell body and ventral dendrites. In 1 to 3 μ sections of brains fixed by perfusion, clubs are in intimate contact with dendrite. At the synapse only a single membrane can be resolved, but there is evidence that each element has a limiting membrane. There is no indication of any intervening 'pericellular terminal net.' In the clubs mitochondria are either uniformly dispersed or aggregated near synaptic membrane. Neurofibrillae (stained with thionin) extend in long axis of dendrite and usually run parallel to membrane. In clubs they are likewise longitudinally arranged and often at right angles to those of dendrite. None observed to cross between the elements of synapse. Tendency of endings to nestle in at base of dendrite branches and occasional spreading of endings over eminences of dendrite make it impossible to interpret 5 μ sections. In Bielschowsky-Rogers preparations it is possible to demonstrate the production of overlay artefacts simulating continuity.

- (1) 13. *Studies on the transmission and recording of auditory impulses from the dog's ear.* T. H. Bast, J. A. Eyster, R. West, O. L. Backus, R. Noer, and M. Krasno, Departments of Anatomy, Physiology, and Speech, University of Wisconsin.

The remarkable phenomenon of the reproduction of voice sounds and musical tones entering the cat's ear, by amplification of electrical currents from the eighth nerve and neighboring structures, first observed by Wever and Bray and confirmed by Davis and Saul, Adrian, Hughson and Crowe, and Witting, was reconfirmed by us on twenty dogs and the sounds were phonographically recorded. We tested the conduction with one electrode on the cut surface of muscle and the other on one of the following structures: Eighth nerve, tenth nerve, cerebellum, cerebrum, bony tentorium, bony crest of the internal auditory meatus, occipital bone, various parts of the middle ear, bony rim of the round window, and external auditory meatus. The conduction was best from the round window and eighth and tenth nerves, and poorest from the external auditory meatus and occipital bone. The effects of plugging the round window, tension on or cutting of the tensor tympany, tension on and cutting of the stapedius muscle, puncturing of the round window membrane and withdrawing fluid, were recorded. Records were also made of sounds transmitted by the dog's ear after the dog was dead. Following the cessation of the heart beat there is a rapid decrease in the intensity within the first 5 minutes, and then a slow decrease until the final disappearance usually about 1 hour after the death of the dog.

- (31) 14. *The circulatory system of urodele larvae as altered by pituitary rudiment implants.* Raymond F. Blount, University of Minnesota.

In *Amblystoma* embryos the gill and tail fin capillaries of triple pituitary animals show constriction as early as stage 43, which is some time before feeding begins. This is a little later than the increase in pigment appears. The reduction in size and number of capillaries and the sequence of change have been determined. This vasoconstriction persists during the larval period. The spleen shows a very definite contraction. The heart is enlarged so that the pericardium is greatly extended with each beat. The rate is slowed and often irregular.

The volume of the ventricular wall as determined from serial section by the paper cutout method shows great increase. In respect to body volume, determined in a similar manner, the increase may be as high as 300 per cent in extreme cases. These circulatory changes are correlated with the reduction in growth of the triple pituitary animal, particularly of the gills, digits, and tail fin. These parts are increased in the hypophysectomized animal in which there is a corresponding vasodilatation.

- (52) 15. *The effects of vitamin E deficient and fat deficient diets on the suprarenal glands of the albino rat.* C. M. Blumenfeld, University of Minnesota. (Introduced by C. B. Freudenberger.)

Size changes of cortex and medulla of the suprarenal glands were studied by the paper outline method in 22 virgin rats, 22 normal pregnant rats, 23 E deficient, pregnant rats, and 24 pregnant rats cured of E deficiency to determine the effects of E deficiency during pregnancy. Similarly studied were the suprarenals of 11 female and 10 male normal rats, 17 female and 9 male fat deficient rats, and 18 female and 6 male rats cured of the symptoms of fat deficiency. The suprarenals of normal pregnant rats showed no increase in size of cortex or medulla. Those of the E deficient, pregnant rats showed a statistically insignificant increase in size of the cortex and a definite atrophy of the medulla. In the pregnant rats cured of vitamin E deficiency the cortex was significantly enlarged, while the medulla had returned to normal size. In the female rats on a fat free diet the cortex and medulla were definitely decreased in size; in male rats they showed a similar trend, but the decrease in size of the cortex was not significant. In the cured rats the medulla of the suprarenals of both male and female rats showed an increase in size, that of the females still remaining subnormal, that of the males returning to normal. The cortex, however, remained practically unchanged.

- (73) 16. *The races of the South Sea, with special reference to skulls of New Britain.* Gerhardt von Bonin, Department of Anatomy, College of Medicine, University of Illinois.

A classification of the races of the South Sea based on the method of the coefficient of racial likeness. To appear in detail in *Biometrika*.

- (81) 17. *The reaction of the human gall bladder to faradic stimulation of the intestinal tract.* Edward A. Boyden, Departments of Anatomy and Roentgenology, University of Minnesota.

Eight medical students, with gall bladders visualized by the Graham method, were subjected to faradic stimulation of the stomach and duodenum.

The instrument used was a Rehfuss tube containing a copper wire soldered to the metal 'olive' at the end of the tube. This apparatus was swallowed to the desired length and its proximal end connected to one terminal of an induction coil. The other terminal was connected with a moist pad fastened to the arm of the patient.

The strength of current used varied from that just producing visceral sensation to that causing intense 'throbbing, spastic pain,' etc.—all sensations being referred to specific areas on the abdominal wall. As tested by the 'barium meal,' such stimulation induced local constriction of the gut. Also the electrode on the arm caused powerful contraction of the underlying skeletal muscle.

Notwithstanding this considerable amount of stimulation, the current failed to inhibit emptying of the gall bladder after a meal of egg yolk. This is in accord with recent observations on monkeys (Proc. Soc. Exp. Biol. and Med., vol. 29, p. 1104), but is in marked contrast to the earlier experiments with cats (Am. J. Physiol., vol. 92, p. 301) and suggests that the evacuating mechanism of the gall bladder of Primates is chiefly hormonal and that contraction is too strong to be inhibited by reflexes originating in the intestinal tract.

- (42) 18. *Observations on the nerve supply of blood vessels.* B. I. Burns, Institute of Neurology, Northwestern University Medical School and Department of Anatomy, Louisiana State University Medical Center.

Serial sections of the femoral saphenous, and anterior tibial arteries were studied, a) in six normal cats and, b) in three cats in which the somatic fibers had been degenerated. After trial of various silver methods, the Ranson pyridine silver method was found to give best results.

The following observations were made:

The farther distal the vessel preparation, the greater the number of nerve fibers.

Comparison of vessels from normal and operated animals gives the impression that somatic fibers in the wall of the vessel are few in number.

In both the operated and normal animals, fibers occur, a) in the adventitia in the form of bundles and as isolated fibers, but do not form a plexus; b) on the outer surface of the media as an intricate plexus; c) in the media as a net. No fibers have been seen in the intima.

Nuclei of Schwann's sheath cells were occasionally seen in the adventitia and on the outer surface of the media, but not within the media.

Sensory endings were occasionally seen in the neighborhood of normal vessels, but no endings of any type were demonstrable within any coat of the vessel wall.

No silver method tried seems adequate for detail study of nerve fibers in the vessel wall. Results obtained by any one method are extremely variable.

- (64) 19. *Histological changes in non-regenerating limbs of x-rayed Amblystoma larvae.* E. G. Butler, Laboratory of Comparative Anatomy, Princeton University.

It has been previously demonstrated that x-radiation in sufficient dosage prevents regeneration of the limb in *Amblystoma* larvae (Butler, '31). This effect occurs regardless of the age of the larvae or the stage of limb development at the time of amputation. Histological study has been made of the non-regenerating limb stumps of radiated larvae in comparison with normally regenerating limbs. Results show that the primary effect of radiation is a complete blocking of the differentiation process within the amputated limb. At the distal end of a non-regenerating limb stump a condensation of cells occurs which resembles

the formation of the blastema in a normally regenerating limb. These cells, however, show no indication of differentiating into the components of a new limb. In normally regenerating limbs the first component of the new limb to be formed from cells of the blastema is cartilage. In radiated larvae not only is there no indication of cartilage formation, but also the old cartilage within the limb stump is seriously affected. Beginning distally, the old cartilage gradually 'melts away' and disappears. This process continues till all of the cartilage within the limb has vanished. The non-regenerating limb of a radiated larva is then a somewhat shrunken stump, completely devoid of cartilage and composed principally of scattered cells which have the general appearance of undifferentiated mesenchyme cells. (See demonstration no. 9.)

- (66) 20. *The transplantation of embryonic mammalian primordia.* Adrian Buyse, School of Medicine and Dentistry, University of Rochester. (Introduced by R. K. Burns, Jr.)

The unusual advantages of the kidney as a new site for transplantation of embryonic primordia, are established as a result of a series of transplantations of posterior halves of 11-day rat embryos, or the mesonephroi and gonad primordia of 12-, 13-, and 14-day embryos, to the kidneys of young or adult hosts.

These primordia are placed in a subcapsular position on the kidneys. They become well established, developing for periods of from 7 to 28 days. Ectodermal structures like skin, nervous tissue, and adrenal medulla, are well differentiated. Among the mesodermal tissues differentiated are connective tissue, cartilage, bone, smooth and striated muscle, metanephros, gonad, and adrenal cortex. Complete intestine with muscle wall and endodermal epithelium also differentiates extensively.

The chief advantages of the kidney site are, ease of transplantation, 80 per cent or more of the grafts becoming established; ready visibility and recovery from the host tissues, from which the graft may be clearly differentiated; rapid and adequate vascularization; and freedom to grow without restriction, the capsule interfering little with the growth of the graft. The optimum period for differentiation is between 14 and 21 days. Tissues fully differentiated at the end of 3 weeks usually undergo a process of resorption. All 4-week grafts show degenerative phenomena.

A special study of sex differentiation in the gonads of these grafts is being made.

- (77) 21. *The ilio-psoas bursa in man.* Simon B. Chandler, Department of Anatomy, Loyola University School of Medicine. (Introduced by R. M. Strong.)

Studies have been carried out on 375 ilio-psoas bursae in 204 cadavers. The size of the bursa varied between 5 to 7 cm. in length and 3 to 4 in width. The bursa communicated with the cavity of the hip joint forty-nine times. This communication or foramen measured from 2 to 30 mm. in diameter. It was bilateral in 12 cadavers and unilateral in 26—14 on the right side and 12 on the left. In most instances, the floor of the bursa contained remnants of bursal tissue and a worn area over the ilium or at its point of contact with the capsule of the hip joint. The capsule varied in thickness, at this point of contact, from 2 mm. to a very thin translucent membrane.

Is this foramen the result of development, or is it produced by the friction of the ilio-psoas tendon? The gross appearance of the foramen, the worn areas and shreds of tissue in floor, suggest wear by the ilio-psoas tendon. The region of the hip joint has been carefully dissected in thirteen fetuses and newborn infants. The capsule has been intact in all cases. The bursa has been present in all the infants, but has not communicated with the capsule in any instance.

- (44) 22. *The endocrine chain in relation to reproduction.* F. E. Chidester, West Virginia University.

In our experiments of the past 7 years, carried on with rats, chicks, tadpoles, and fruit flies, we have accumulated evidence, which, combined with other reported work, has led to the formulation of a working hypothesis regarding the role of the endocrines as reservoirs of iodine.

Our 1929 studies indicated that suprarenal cortex induces prematurity in chicks and rats. More recently, we have found that anterior pituitary extract, like pregnancy urine hormone, may induce Graves disease, and even cause rickets, by the iodine action. Analyses by Smith, Closs, and others show the iodine content of anterior pituitary to be high. Similarly, Gley found that parathyroids were rich in iodine.

Histologically, the pineal, parathyroids, thymus, anterior pituitary, adrenal cortex, interstitial cells, and corpus luteum could retain iodine. Data from tadpole experiments and actual published chemical analyses indicate that these organs do have iodine at some period in their existence.

The pineal could act as a reservoir protecting against prematurity, succeeded by the thymus. Then the pituitary, and parathyroids could function during later life. The corpus luteum of pregnancy, acting as a reservoir for excess thyroid-iodine, would inhibit ovulations. But profound emotional disturbance might permit ovulations and superfetation.

Butenandt's hormone, highly unsaturated, might activate the thyroids just as unsaturated fatty acids do in experimental studies.

- (39) 23. *Components of the nerves to the nasal mucosa.* Kermit Christensen, St. Louis University School of Medicine. (Introduced by Albert Kuntz.)

The components of the nerves to the nasal mucosa, in the cat, are derived mainly from three sources, viz., the nerve of the pterygoid canal, the sphenopalatine nerves, and the sphenopalatine ganglion. The nerve of the pterygoid canal includes large, medium-sized and small myelinated and many unmyelinated fibers. The small myelinated fibers synapse in the ganglion. The others pass through the ganglion to become incorporated in nasal nerves. The sphenopalatine nerves consist of large and medium-sized myelinated fibers and a few unmyelinated fibers. These also traverse the ganglion. The bulk of the fibers contributed by the ganglion are unmyelinated. The nasal nerves are composed of large and medium-sized myelinated fibers and unmyelinated fibers from these sources.

- (5) 24. *Microscopic observations in the living rabbit of the new growth of nerves and the establishment of nerve-controlled contractions of newly formed arterioles.* E. R. Clark, E. L. Clark, and R. G. Williams, Department of Anatomy, University of Pennsylvania.

In the new tissue which grows into the space left in the 'round-table' chambers inserted in the rabbit's ear, a variable number of nerves may grow. It is possible to see clearly without vital dyes the medullary sheath, and the sheath cells, and already interesting studies of myelination have been completed. By an adaptation of the use of methylene blue, temporary staining of the axones has enabled us to see the non-myelinated nerves.

It is also possible to see clearly the newly developed arterioles, to distinguish nerve-controlled contractions, and to watch their gradual outward extension.

By a combination of the two, we have succeeded in demonstrating, on three successive stages in the same animal, the progressive and coextensive side by side development of nerve-controlled contraction in an arteriole, and of non-myelinated nerves.

This observation proves conclusively that the nerves which control the contraction of the muscle cells on arterioles may undergo new growth at the periphery in the adult animal, and may reestablish nerve control over vessel contraction.

- (8) 25. *A form of congenital myotonia in goats.* Sam L. Clark, Frank H. Luton, and Jessie T. Cutler, Departments of Anatomy, Medicine, and Pharmacology, Vanderbilt University School of Medicine.

Goats of a distinct breed occurring in middle Tennessee and adjacent regions exhibit a myotonic state when suddenly excited, surprised, or when faced with the necessity of quick action in response to an environmental situation. Under such circumstances the voluntary muscle of the goat becomes rigidly contracted, with the limbs in extension, and muscles of the trunk so contracted that the position of the goat is similar to that of decerebrate rigidity. Such spasms last from a few seconds to more than a minute, during which time the goat may be pushed over or handled as if it were a wooden image, and voluntary activity is not possible. The muscles gradually relax and voluntary movements return, though limited at first. The condition is apparently hereditary. Attempts to discover the underlying mechanism have been directed toward the chemistry of the blood, with especial attention to its content of sugar, calcium, and inorganic phosphates. Analyses have been made of the blood of animals in a resting state, as well as following excitement or exercise, and after the injection of glucose, adrenalin, and insulin. The results of these compared with similar experiments on normal goats showed no significant variations. Further experiments are under way which will attempt to trace the neural mechanism underlying the myotonia.

- (71) 26. *Integumentary asymmetries in situs inversus viscerum totalis*. Harold Cummins, Department of Anatomy, Tulane University.

The palmar dermatoglyphics and finger-tip patterns are analyzed in nine cases of complete visceral transposition for evidences of asymmetry reversal. The findings are negative in that no departures from characteristic variants of these features are demonstrable. It is to be emphasized that the dermatoglyphic asymmetry which represents the normal control for these cases is highly variable in the individual instance. The present series, with dermatoglyphic variants which are matched in normals, therefore only serves to show that if any dermatoglyphic indication of reversed asymmetry is to be obtained at all it must be sought in statistical trends rather than in the individual case, which is considered negative, since aberrant asymmetries are lacking. Absence of reversal in the crown hair whorl is indicated by the occurrence in seven cases of a clockwise whorl, centrally situated or slightly shifted to the right, with but two cases of counterclockwise direction—a proportion agreeing with normal variation. It may be concluded that asymmetries of dermatoglyphics and crown hair whorl are conditioned independently of visceral asymmetry.

- (37) 27. *Cells and fibers in spinal nerves, C2, C6, T4, T9, L3, S2, and S5 in man*. H. A. Davenport and R. T. Bothe, Northwestern University.

Enumerations of nerve fibers in dorsal roots, cells in dorsal root ganglia, fibers in ventral roots, and fibers in spinal nerve trunks just distal to the ganglia were made. The number of axis cylinders in the dorsal root to each 100 cells in its ganglion varied from 78 to 102. The nearest approach to a 1:1 ratio was found in C6, with 102, and L3, with 97. The number of unmyelinated fibers in the dorsal root was greatest in T9 (1 myelinated to 1.76 unmyelinated) and least in C2 (1 to 0.52). The proportion of unmyelinated fibers both in dorsal and ventral roots was higher in thoracic and sacral than in cervical and lumbar nerves. The sum of all fibers in both roots equaled the number in the spinal nerve trunk in C6, T4, L3, and S2. More myelinated fibers were found in the trunk than in the combined dorsal and ventral roots in C6, T9, L3, and S2.

- (90) 28. *A circumvallate placenta in utero*. Marie Dees-Mattingly, Department of Anatomy, Tulane University.

The placenta here described is typically circumvallate, according to Williams ('27). Rarely is it possible to examine this infrequent form of placenta in situ. The present specimen was removed, prophylactically, from a tubercular patient at the fifth month of gestation.

The ring, which varies in width, height, distance from periphery of the placenta, and depth of the furrow under its fold, divides the placenta in two parts—chorial and extra-chorial portions. The chorion frondosum terminates at or near the inner edge of the fold. The fold, composed largely of necrotic tissue, decidual cells and rudimentary villi, includes in order outward from the free inner surface: amnion, chorion laeve, decidua capsularis, decidua vera, chorion frondosum, and amnion. Fetal membranes abandon the placenta at the outer margin of this amnio-chorial duplication, leaving the extra-chorionic portion of

the placenta covered by decidua. In the vicinity of the fold, uterine glands are greatly augmented, thereby increasing the thickness of the decidua, comparatively thin in the main part of the placenta.

In the absence of tangible evidence concerning implantation, it is impossible to state conclusively the etiology of circumvallate placenta. However, the large villus roots with the many small branches dispersed through a relatively small, thick placenta, and the extra-chorionic structural relations are plausible accompaniments of undue restriction of the chorion frondosum in the early differentiation of the chorionic vesicle.

- (75) 29. *The behavior of the epiphyseal cartilages in rachitic rats.* G. S. Dodds, Medical School, and H. C. Cameron, Agricultural Experiment Station, West Virginia University.

Albino rats when 4 weeks old were put upon a high calcium, low phosphorus diet (Steenbock-Black diet 2965). Rickets developed promptly. Leg bones elongated about as in controls for 2 weeks. After 3 weeks they grew no more.

The epiphyseal cartilage in the head of the tibia showed the following: It thickened about fivefold in 3 weeks, then ceased to grow. The zone of cell division was about normal for 3 weeks, after which mitoses were rarely seen. The flat cells in the columns enlarged normally at first, but soon attained an average length of 16μ or less, compared with 25μ in normals. Limited growth and crushing of cells caused the difference. Calcification ceased during the first week. Cartilage removal stopped at the limit of calcification. The zone of large cells became very thick. Cell columns were well maintained and became very long, increasing from about 4 large cells to 75 to 140. Cell columns were of normal width. Cartilage removal was soon renewed, but in an irregular and atypical manner which left long tongues and many isolated masses of cartilage in the metaphysis. This process by the eighth experimental week had reduced much of the cartilage to about normal thickness. The cartilages in other bones of the legs behaved substantially as described above. (See demonstration no. 10.)

- (80) 30. *Direct observation of the contraction of the gall bladder in the opossum.* Franklin S. DuBois and Gene H. Kistler, Departments of Anatomy and Physiology, University of Alabama. (Introduced by E. A. Boyden.)

Contraction of the gall bladder in the opossum has been consistently obtained by stimulating the surface of the vesicle with a faradic current. When such a stimulus is applied to the corpus or fundus, a localized shallow constriction appears. Frequently there are small rounded protuberances in the wall. As expulsion of bile occurs, the transparent wall becomes bleached, opaque, and eventually puckered. Repeated electrical stimulation may result in complete emptying of the viscus.

After the common bile duct has been severed at its upper end—to eliminate any possible evacuating action of peristalsis of the duct—stimulation of the gall bladder causes as great a discharge of cystic bile as before. During such periods of contraction, increase of intravesicular pressure—as measured by a manometer of 1 mm. bore connected to the cystic duct—registers as high as 15 to 40 mm. of water.

Stimulation of that portion of the gall bladder adjacent to the liver produces little alteration in the appearance of the wall of the vesicle and no change in intravesicular pressure. Serial sections of gall bladders fixed in 10 per cent formalin during the height of contraction, demonstrate that the smooth muscle layer is thicker in the peritoneal than in the hepatic portion of the gall bladder wall.

- (88) 31. *Functional localization in the renal tubule of the frog.* J. Graham Edwards, Medical School, University of Buffalo.

Studies on the excretion of iron by the normal kidney and as affected by diuretics have not revealed a functional differentiation within the proximal convolution. If, however, the frog is given bichloride of mercury, potassium chromate or uranium nitrate either along with ferric ammonium citrate or the latter is given 12 hours later, a differential susceptibility of the cells of the proximal convolution to the poisons is segmentally manifest. The iron is deposited in the cells in greatest amounts depending on the degree of the necrosis of the cells of a given segment. This is in contrast with the action of urethane which causes marked increase in the intracellular content of iron without obvious necrotic changes in the cells.

The content and segmental localization of iron in the tubule varies, depending on whether a mixture of iron salts or of ferrocyanide alone is given with the poisons mentioned.

Quantitative determinations of the excretion of ferric iron under the influence of urethane, caffeine, and potassium chromate show that these are diuretic for iron, whereas bichloride of mercury is not.

- (26) 32. *Biological responses of the female macacus monkey to the A-P-L hormone of human pregnancy urine.* Earl T. Engle, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Follicular growth is not caused by the A-P-L factor from pregnancy urine even with massive doses or long treatment and since no reddening of the sex skin occurs, which is a delicate indicator for oestrin, it is assumed that oestrin is not produced as a result of treatment (Engle, '32, '33).

Uterine bleeding in mature animals occurs after the removal of a single ovary, but this is not true of younger (ca. 3000 gm.) animals (Allen, Engle). The oestrin production of young animals is not sufficient to cause uterine bleeding upon its diminution or withdrawal.

Treatment of sub-adult monkeys (2400 to 3500 gm.) with pregnancy urine extract usually results in uterine bleeding, macroscopic in degree, though never profuse. This bleeding may continue for 14 days during continued treatment. In certain animals the interval endometrium is substantially intact, the vessels congested with sub-epithelial haematomas. In other animals there is extensive tissue loss after 10 days of bleeding.

Whereas it has not been proved that oestrin withdrawal is not the initiating factor, it is provisionally considered that this type of experimental bleeding is due to the action of the pregnancy urine factor, on muscular tone and by the

passive vascular congestion. This active principle, which biologically is not the same as the anterior pituitary factor, is not a part of the physiological economy of the macacus, even during pregnancy, and as used, is an extraneous principle.

- (70) *33. Resolution of dissecting-room material into human types.* Thomas Horace Evans, Long Island College of Medicine.

By recognizing the concomitant production of several more or less primitive features, a man, or men, of types which may be provisionally styled 'primitive' may be attempted.

The ent-epicondylar process, the metopic suture, the muscular so-called reversions, the opening between the cavum of knee-joint and the superior tibio-fibular cavum, the facets of bones of the foot, and the contact of calcaneum with os naviculare, the contact of the third cuneiforme with the side of base of the fourth metapodial, and the cuboido-navicular facet may be included.

Attachment of the left anterior papillary muscle of the right ventricle not to the septum, but to anterior wall noted, as a reptilio-marsupial vestige. (See demonstration no. 12.)

- (13) *34. Studies on the vagus nerve of the cat.* James O. Foley and Franklin S. DuBois, Department of Anatomy, University of Alabama.

The rootlets of the right vagus nerve were sectioned intracranially in two cats. Subsequently the medulla oblongata of each animal was prepared by the Marchi method. In these stained sections motor, sensory and mixed sensory motor fascicles have been identified.

The right nodose ganglion was removed in six other cats. In five of these, serial sections of the medulla were prepared by the Marchi method, while in the sixth the rootlets and the trunk of the vagus nerve above the point of severance were stained by the pyridine silver technique. It was found in these preparations that the majority of the nodose fibers pass through one side of the jugular ganglion without traversing the region occupied by cells, as currently described by Ranson in the normal vagus. Cephalad of this ganglion mingling of jugular and nodose fibers occurs. Although this mixture is rather marked as the rootlets enter the brain stem, some few of the jugular fascicles remain discrete and lie dorsal to the mixed fascicles as they pass medialward in the medulla. At the lateral border of the tractus solitarius most of these dorsally situated jugular bundles join the mixed jugular and nodose bundles and enter the ventral portion of the tract. An occasional one, however, maintains its separate course and appears to terminate more dorsally in the tract.

- (82) *35. Observations on the anatomy and function of the gall bladder in children.* Alice H. Fuller and Edward A. Boyden, Departments of Pediatrics and Anatomy, University of Minnesota.

The present study is based upon a detailed roentgenologic examination of sixteen boys and twelve girls between the ages of 6 and 11 years, each case involving some fifteen cholecystograms made at periodic intervals after a standard meal of egg-yolk.

Owing to the relative thinness of the trunk in children, it was possible to visualize the cystic and common ducts in about half the cases; also to x-ray the gall bladder through the side as well as through the back, thereby obtaining new information regarding the antero-posterior diameter and orientation of vesicle and ducts in the living body. Thus it may be stated that the long axis of the gall bladder when the child is prone forms an angle of about 20° with the vertebral column, but in erect posture this angle is almost obliterated.

It is of special interest that the antero-posterior diameter diminishes after food at the same rate as the frontal diameter. Using the latter as a basis for computation, it was found that the gall bladders of the first sixteen children studied (the other figures are not yet available) emptied faster than those of eighteen college students previously studied. Although this difference is not great enough to be statistically significant, these figures are probably not without significance when one considers the more rapid passage of food through the small intestine in children. (See demonstration no. 6.)

- (63) 36. *The structural appearances of unstained, living, fresh, and fixed tissues and organs revealed by the bright-field, the dark-field, the ultra-violet, and the polarizing microscope in comparison with stained and with incinerated tissues and organs.* Simon H. Gage, Mary Gage Day, and Clara C. Starrett, Cornell University.

In the investigation here reported, the effort has been made to gain a more complete understanding of the activity, structural composition and relations, and the physical reactions of bodily structure than can be obtained by any single method of approach.

As will be shown by accompanying diagrams, each method gives a different picture of the same thing; each picture is therefore only a partial representation. A mental combination of them all gives a composite more nearly like the actual thing than any single representation.

The investigation gives, furthermore, a renewed emphasis to the fact that even with these multiple view-points, our understanding of the real structure of living tissues and organs is still appallingly incomplete.

- (49) 37. *Cellular constituents of the anterior pituitary after uterine implants of carcinoma in rats.* M. F. Guyer and P. E. Claus, University of Wisconsin.

Our earlier studies revealed marked increase in the number of basophile cells in the anterior lobe of the hypophysis after subcutaneous implantation of carcinoma, with no detectable change in the numbers of the oxyphile cells. The present study shows that implantation of such tumor tissue in the uterus instead of subcutaneously, results in a noticeable increase in the number of oxyphile cells and a less marked increase in the basophiles of the anterior lobe. Since pregnancy produces a similar increase in oxyphiles, these additional cells may correspond to the so-called pregnancy cells. In rats with such uterine cancer, however, the estrual cycle continues and sections of the ovaries disclose the usual histological picture of the non-pregnant rat. (See demonstration no. 14.)

- (65) 38. *Regional differences in the organization center of the amphibian embryo.* Edmund K. Hall, Department of Anatomy, School of Medicine, University of Louisville. (Introduced by S. I. Kornhauser.)

Spemann has shown that the organization center of the amphibian embryo contains regional differences: 1) The anterior portion may be designated as 'head organizer,' for it induces the formation of a brain from any part of the presumptive epidermis of the gastrula. 2) The posterior portion may be called 'trunk organizer.' It induces a spinal cord from the presumptive epidermis of the trunk, but, in the head region, it induces a brain.

How will these regions of the organization center affect the ectoderm of the presumptive neural plate? The results show that when head organizer is allowed to act upon the ectoderm of the future spinal cord, a normal cord forms. The action of head organizer is, therefore, not specific under the conditions of the experiment. Trunk organizer, on the other hand, acting upon the presumptive ectoderm of the brain, brings about the formation of a very abnormal head, in which the neural tube is elongated and narrow, and the characteristic head organs are absent. Evidently, under these conditions, the level of the embryo at which the graft is acting does not determine the type of reaction to trunk organizer.

- (79) 39. *An inquiry into food as a factor in hepatic rhythm.* George M. Higgins, Joseph Berkson, and Eunice Flock, Division of Experimental Medicine, The Mayo Clinic.

A daily rhythm in its glycogen-forming and bile-secreting functions exists in the liver. Glycogen is high at night and low during the day; while bile production is low at night and high during the day. The predominant opinion in the literature is that this rhythm is independent of nutritional factors.

When rats were fed from 9:00 to 11:00 in the morning the curve of the liver changes during the following 24 hours was bimodal, peaks occurring at 5:00 to 7:00 P.M. and 11:00 to 1:00 A.M. When rats were trained to eat from 3:00 to 5:00 P.M. and killed at 2-hour intervals for 24 hours, a similar bimodal curve in liver changes occurred. The peaks in the curve had shifted corresponding with the shift in the feeding period.

Rats were trained to eat and drink for 2 hours progressively for 24 hours. Ten rats were trained to eat from 9:00 to 11:00 A.M.; ten from 11:00 to 1:00 P.M.; ten from 1:00 to 3:00 P.M., etc. After a 10-day training period all rats were killed 6 hours after eating 10 grams of food. In preliminary training, duration of feeding, amount of food consumed and time of killing after feeding, all rats were alike. The only varying factor was the time the animals were fed. Under these conditions, the curve of changes in the weight of the liver, its glycogen and water content was practically a straight line.

- (27) 40. A refractory condition in the ovaries of the *Macacus rhesus* monkeys and rabbits after continued hypophyseal stimulation. F. L. Hisaw, R. Hertz, A. Hellbaum, and H. L. Fevold, University of Wisconsin. (Introduced by M. F. Guyer.)

Daily subcutaneous injections of the water soluble fraction of a pyridine extract of dried sheep pituitary were given for extended periods to juvenile and adult female *Macacus rhesus* monkeys and to 12-week-old female rabbits. The daily dosage for the monkeys was the equivalent of 1 gm. of dried tissue; the rabbits received half this amount.

After about 8 to 10 days of treatment a maximal response is obtained. From this point on, continued injections at the same dosage are unable to maintain the ovaries in this highly stimulated condition. The ovaries resume their previous gross and histological features. In the monkey, menstruation is associated with this ovarian regression. The ovaries continue to be refractory to further hypophyseal stimulation even when injections are continued for as long as 50 days in the monkey and 25 days in the rabbit.

- (53) 41. *The lipoids of the adrenal.* N. L. Hoerr, Department of Anatomy, University of Chicago. (Introduced by B. C. H. Harvey.)

The adrenal cortex of the guinea pig is particularly rich in cholesterol esters, oleic acid, and lecithin. No definite relationship has been established between chemical analyses and histological picture. None of the present histological methods for differential demonstration of lipoids can be considered as valid microchemical tests. An analysis of these lipid droplets by the best cytological methods yields no substantiation for any of the proposed theories of the functional role of the cortical lipoids. With adequate preservation as also in careful study of fresh tissue the liposomes are spherical and of practically equal size in any one cell, though differing in size from those of neighboring cells. They have no relation to the mitochondria and Golgi apparatus except that of proximity. There is in addition to the visible liposomes considerable masked lipid in the cytoplasm as proved by chemical extraction from tissue which shows no lipid droplets remaining in the sections. So far all attempts to demonstrate this masked lipid histologically in adrenal as in other tissues are microchemically worthless. The sizes of the lipid droplets can be changed by pressure, by unsatisfactory fixation, by lipase activity, and by various experimental variations of lipid metabolism. The amount and character of the lipid can be altered by inanition, experimental scurvy, the injection of toxins, thyroidectomy, chloroform narcosis, pregnancy, lipid feeding, and other experimental procedures.

- (16) 42. *The state of development of the central nervous system of a 16-mm. human embryo, Homo no. 1, Pittsburgh, 1932.* Ira D. Hogg, Department of Anatomy, University of Pittsburgh School of Medicine.

The tractus solitarius and spinal tract of the trigeminal nerve are very distinct; the posterior funiculus of the spinal cord is small and is still further reduced at the caudal end of the medulla oblongata. Differentiation of second order neuroblasts is more pronounced in the nucleus cuneatus than in the posterior

horn of the spinal cord. Most second order neuroblasts are commissural and their fibers turn rostrad. Those of the spinal cord form well-marked ventral and lateral funiculi, which are greatly reduced at the caudal end of the medulla oblongata. Fibers from second order neuroblasts can be traced from the trigeminal nuclei to the rostral portion of the midbrain.

The fasciculus longitudinalis medialis can be traced from the midbrain to the caudal end of the facial nucleus or further caudad. Motor fibers of all nerves have grown into the anlagen of the organs they are to innervate. Cell groupings are beginning to be in evidence in the spinal cord and other motor nuclei.

Differentiation in the diencephalon is limited to the basal portion, that in the telencephalon occurs in association with the olfactory nerves. The cerebellum exhibits very little differentiation. (See demonstration no. 16.)

- (17) 43. *Human fetal activity.* Davenport Hooker, Department of Anatomy, University of Pittsburgh School of Medicine.

In cooperation with various institutions, a study of the activities of living, but non-viable, human fetuses, removed for therapeutic reasons, is being made. The study is intended to supplement the work of Minkowski (human) and to continue the investigations of Coghill (Amblystoma) and Angulo (rat) on the correlated physiological-anatomical phenomena of development of the nervous system.

Four fetuses have been studied physiologically, the ages being approximate only. A 35-day fetus was non-motile. A 60-day fetus showed no spontaneous movements, but responded to tactile stimulation of the cervical region by lateral body flexion. A 3½-month fetus showed spontaneous movements and, in response to tactile stimulation, ventral head flexion, general body reactions, including the limbs, and some specific limb responses. Pressure stimulation caused direct muscle contraction. A 4½-month fetus moved vigorously, both spontaneously and on tactile stimulation. In addition to the response types seen in the 3½-month fetus, the 4½-month fetus showed wrist and ankle movements on stimulation, respectively, of the palms and soles. A vigorous thigh flexion followed reflexly on stretching of the abdominal musculature.

These results should be evaluated cautiously because of the small number of cases and the asphyxiatory conditions of the experiments. Very definite is the easy fatiguing shown by all the fetuses observed. (See also, demonstration no. 17.)

- (2) 44. *Absence of the organ of Corti and its possible relation to electric auditory nerve responses.* Howard A. Howe and Stacy R. Guild, Otological Research Laboratory, Johns Hopkins University School of Medicine.

Already well recognized is the histopathology of the inner ears of certain incompletely albinotic animals which fail to react to sound stimuli, although gait and equilibrium appear normal. Cats of this type, clinically known to be deaf, have been tested for sound transmission by the Wever-Bray technique. In no instance did stimulation of the ear by tones give rise to any changes of electric potential which could be detected by leads from the auditory nerves. The histological picture in the ears of the animals thus tested agrees in essence with that found by earlier observers. There was practically complete absence of the organ of Corti with all of its hair cells, marked pathological changes in the macula

sacculi, collapse of the cochlear duct and sacculus. On the contrary, the semi-circular canals, ampullae, utriculus, and perilymphatic spaces were normal. The number of nerve fibers and cells in the spiral ganglion was slightly reduced, but in no way commensurate with the complete failure of sound transmission. Conversely, this lack of response to tones has never been encountered in experiments where the organ of Corti was demonstrable. The authors see in this experiment of nature an extremely potent answer to the criticism that audio-electric phenomena are produced by vibrations of electrodes or microphonic effects of the middle ear and cochlea, rather than by sensory end organs.

- (68) 45. *Transplantation of the intermediate segment and adjacent mesoderm in the wood-frog (*Rana sylvatica*): its effect upon the sex differentiation of the gonad.* R. R. Humphrey, Department of Anatomy, University of Buffalo.

In the Anura the primordial germ cells during early embryonic life are included in the entoderm of the roof of the archenteron, from which they become separated during the formation of the mesentery. They are subsequently shifted laterally to form the genital ridges. During their separation from the entoderm and 'migration' to the genital ridges they become associated with that lateral and intermediate mesoderm which gives rise to the stroma, rete cords, and covering epithelium of the gonad.

When the intermediate segment and adjacent mesoderm are replaced by those of another embryo before the germ cells have been separated from the entoderm, these cells are later shifted to the operated side in the usual fashion, and thus brought into association with the implanted mesoderm. When donor and host are unlike in sex, the gonad on the operated side differentiates according to the sex of the donor. The primordial germ cells contributed to the gonad by the host entoderm are unable to alter or counteract the genetic factors carried in the implanted mesoderm, and apparently play only a passive role in the differentiation of sex.

When the two gonads in any grafted animal are unlike in sex type, the testis is commonly normal, while the ovary shows some degree of retardation, degeneration, or sex reversal (animals killed at metamorphosis).

- (35) 46. *Differential counts of sympathetic ganglion cells in the rat and the rabbit.* Everett H. Ingersoll, Department of Anatomy, Medical College of Virginia. (Introduced by H. L. Osterud.)

Coeliac ganglion cells, removed from healthy, undisturbed animals, were classified into types based upon the morphological characteristics of the Nissl substance and upon the nucleus-plasma ratio. Counts were made of over 4000 of these cells, with an average of over 400 cells from each animal.

More than one-half of the cells classified in the rats were in the normal and hyperchromatic types, while less than 20 per cent were dechromatized and exhausted cells. Approximately 30 per cent were in types characterized by perinuclear or peripheral bands of chromidial substance.

In the rabbits about one-half of the cells, on the average, had a peripheral band of Nissl substance. The percentage of cells in the normal and hyperchromatic types was less than in the rat, while the number of cells in the de-

chromatized and exhausted types remained about the same. Cells with eccentrically placed nuclei were more common in the rabbit than in the rat. Also binucleate cells, seen only rarely in the rat, comprised nearly 20 per cent of all classified cells in the rabbit. Cells with a perinuclear arrangement of the chromidial substance were practically non-existent in the rabbit. (See demonstration no. 18.)

- (4) *47. The relation of the red nucleus to postural tonus and righting reactions.*
W. R. Ingram, Institute of Neurology, Northwestern University Medical School.

Study of a series of cats subjected to total or partial destruction of the red nuclei by use of the Horsley-Clarke instrument yields evidence that in such animals labyrinthine and body righting reactions may remain functional. These cats show abnormalities of gait which resemble in some degree those occurring in decerebellate animals: dysmetria, adduction of fore limbs, circumduction of hind limbs, rotation of pelvis, etc. Increased muscle tonus is especially manifest under certain conditions, as when supine or suspended, with markedly enhanced resistance to positive stutz stimulation. Certain of the postural reactions as analyzed by Rademaker (Schunkel, Stemmbein and Hinkebein reactions) may be deficient in the acute stages, but soon merely show evidence of the delay typical in decerebellate animals.

Bilateral labyrinthectomy in such cats no more affects their ability to maintain an upright position when on a supporting surface than it does in otherwise normal animals, nor does it affect the special stutz and tonus reactions appreciably.

Destruction of the red nuclei in chronic bilaterally labyrinthectomized cats with good compensation induces typical disorders of locomotion, increase in the stutz and tonus reactions, and delay in the postural reactions. Body on the body, and body on the head righting reactions are retained, and such animals are capable of proper orientation when on a solid supporting surface.

- (84) *48. The weights of the various organs in adult rats after inanition with or without the dietary accessories.* C. M. Jackson and C. E. McLennan, University of Minnesota.

Sixty male Wistar albino rats weighing 240 gm. each were divided into four groups of fifteen each. The normal controls were killed immediately; the three test groups after loss of 40 per cent in body weight. On water alone, the time averaged 12.27 days, and the organ weights were classified as follows: 1) Brain, spinal cord, eyeballs, skeleton, suprarenals, and stomach remained nearly constant. 2) Head, testes, epididymides, lungs, integument, hypophysis, and kidneys lost relatively less than the body. 3) Thyroid and submaxillary glands, heart, musculature, and prostate decreased proportionally to the body. 4) Intestines (also contents), spleen, pancreas, liver, and thymus lost relatively more than the body.

In another group, fed merely the normal accessories (protein, salts, vitamins, and water), the duration was 20.27 days. The fourth group, fed a starch-sugar mixture of equivalent calories, required 17.33 days (difference not statistically significant). Therefore, the dietary accessories as such did not essentially modify the rate of loss in body weight.

The organ weights were generally similar in the test groups. In those fed accessories, smaller losses in the pancreas, submaxillary glands, kidneys, and gastro-intestinal contents may indicate a protective influence, but greater losses occurred in the stomach and testes. Both accessory and energy tests (in comparison with those on water only) showed somewhat greater losses in the thymus, musculature, and heart, perhaps due to the longer inanition.

- (45) 49. *The form of the seminiferous tubule in man.* Franklin P. Johnson, University of Oregon.

By means of maceration in acid the contorted seminiferous tubules of the adult human testis have been dissected. The dissections show conclusively that the tubules, for the most part, have the form of loops, one loop connecting with one or more others, to form a series of loops of undetermined extent. Cross anastomoses have also been found. Tubules with blind ends are also present, but are not as numerous as the loops. Diverticula of the seminiferous tubules, which Sappey described in 1874, and which Bremer failed to find in human fetal material and Huber and Curtis in the rabbit, are not of uncommon occurrence.

- (74) 50. *Studies on bone growth as seen in the transparent chamber introduced into the rabbit's ear.* Henry T. Kirby-Smith, Department of Anatomy, University of Pennsylvania. (Introduced by E. R. Clark.)

Following transplantation of 6-day callus into a round table chamber introduced into the rabbit's ear, two masses of new bones differentiated and their behavior was studied microscopically for several weeks.

Following partial absorption, a miniature fracture occurred, and the sequence of events observed, with the following chief results:

After 2 days, new cells had migrated from the bone and fibers had appeared, both of which, but particularly the fibers, increased during the next 3 days. On the sixth day refractile granules appeared throughout the fibers. These had increased in size on the seventh day and by the ninth had coalesced, forming a homogeneous highly refractile material with lacunae—a typical picture of new bone.

The separated fragment was absorbed between the sixth and eighth days.

There was no appreciable change in either the blood-vessel pattern or the circulation during the processes of growth and resorption.

The tempo of this new formation of bone following a fracture of microscopic size was about the same as that for the series of events following injury to the rabbit's ulna or radius.

- (86) 51. *Malpighian tubules studied by intravital dyes.* S. I. Kornhauser, Department of Anatomy, School of Medicine, University of Louisville.

The malpighian tubules of *Anisolabis* were studied in hanging drop with oil immersion, being taken, after intervals of 5 minutes to 2 hours, from animals first anaesthetized in CO₂ and then hypodermically injected with vital dyes. Neutral fuchsin was one of the best dyes. It clearly showed the tubules to be composed of three parts, a terminal or distal thin walled portion which did

not take up any coloring matter, a medium heavy walled portion (the longest part) which has a great affinity for both acid and basic dyes, and a proximal section in the lumen of which the dye was concentrated and appeared in granular form. The distal portion often bulged and appeared to be a water absorbing region. It generally collapsed on fixation. The chief cells of the middle tube contain many vacuoles which have a great affinity for basic dyes and are blackened by osmic acid. These cells have tall brush borders, which do not move like cilia in propelling particles toward the proximal portion. Between the chief cells there was demonstrated, by methylene blue and similar stains, accessory cells of entirely different form and staining reaction. These are probably phagocytic and do not possess brush borders. The proximal tube has a wall of medium thickness devoid of accessory cells and appears to be utilized in the return of water to the coelom.

- (41) *52. Origin of nerve fibers associated with the common, internal, and external carotid arteries.* Albert Kuntz, St. Louis University School of Medicine.

Extirpation of the superior cervical sympathetic ganglion, in the cat, does not result in degeneration of all the nerve fibers which extend into the cephalic region along the internal and external carotid arteries. By means of experimental operations, involving extirpation of the inferior cervical ganglion in some animals and extirpation of the nodose ganglion in others, it has been determined that the fibers extending cephalad along the internal and external carotid arteries, which remain intact following extirpation of the superior cervical sympathetic ganglion, comprise components of the vagus and upper thoracic nerves.

- (9) *53. The fundamental divisions of the cerebellum.* O. Larsell, University of Oregon Medical School.

The basic cerebellar parts are the vestibulo-lateral auricular lobe and the predominantly trigemino-spinal corpus cerebelli. In land forms the auricular lobe becomes relatively reduced in size, while the corpus cerebelli increases greatly, so as to quite obscure, in some forms, the auricle. In turtles and alligator the auricle, now better called the floccular lobe, can be recognized by surface markings and fiber connections. In alligators the floccular lobes fuse beneath the corpus cerebelli and are bounded from the latter by a fissure, the first to appear.

The corpus cerebelli arches upward and forward in lizards, upward and caudally in other reptiles. In the alligator the caudal portion bends downward so that the whole forms a dome. Continued increase in volume results in three primary transverse bands with fissures between. The two anterior bands correspond to anterior and median lobes of authors. The posterior band is separated from the floccular lobe by a morphologically more primitive fissure, and should not be included with the floccular lobes as the posterior cerebellar lobe. All three bands are secondary features as compared with corpus cerebelli and lobus floccularis.

In mammals the primary bands undergo further subdivision, but the first two fissures become the most marked. The bilaterally paired corpora cerebelli and floccular lobes represent more fundamental subdivisions than do the anterior, median, and posterior lobes.

- (85) 54. *Measurements of the skull and of some of the long bones of the muskrat.* Homer B. Latimer and Ray B. Riley, Department of Anatomy, University of Kansas.

Skeletons of 124 muskrats from the collections of the University Museum were used in this study. The lengths of five pairs of long bones and twelve dimensions of the skull were measured with a vernier caliper reading to tenths of a millimeter. The long bones, the skull, and the mandible were weighed on a chemical balance sensitive to tenths of a milligram. The lengths and weights of each bone of each pair of the long bones were determined separately.

The left bone of each pair was longer in the greatest number of cases, followed by the right member of the pair, and the two bones were of equal length least frequently. The same is true for the weights of the bones, except for the tibia, and in this case the right tibia was heaviest most frequently.

This material will be presented statistically at the meeting.

- (83) 55. *The effect of castration, with and without removal of the epididymis, in the rat.* J. J. Lawless, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.)

Thirty-one albino rats were castrated at the age of 21 days, twenty litter mates serving as controls. In twelve animals both the testes and epididymides were removed. Only the testes were removed in the remaining nineteen. All operations were performed aseptically, under ether anesthesia, by the same operator. Controls were subjected to similar operations, except that testes were not removed. Complete autopsy was done on all animals at the age of about 100 days, all organs being weighed and preserved. Body weight and body length are slightly reduced in the test animals. Skeletal growth, as measured by length and weight of the femur, is decreased. The organs showing an increase in weight are thymus, adrenals, and hypophysis (anterior lobe only, Stein). Those showing a decrease in weight are accessory reproductive organs (prostate, seminal vesicles, etc.), kidney, liver, pancreas, and heart. The organs which are not changed in weight are the pineal body, thyroid, brain, and spleen. In general, both groups of castrated animals show the same changes in organ weights. The lumen of the duct of the epididymis is reduced in size and is empty. A relative increase in connective tissue is noted in the epididymis. In the ductus deferens there is a reduction in thickness of the muscularis and an apparent stratification and reduction in height of the epithelium.

- (72) 56. *Dermatoglyphics and functional lateral dominance in Mexican Indians (Mayas and Tarahumaras).* Stella M. Leche, Department of Anatomy, Tulane University. (Introduced by Harold Cummins.)

The dermatoglyphs of two groups of Mexican Indians were studied in conjunction with the functional lateral dominance of each subject. The number of Indians studied in each series is small, but, in spite of this fact, certain dermatoglyphic features (notably, the proximal direction of a line A; the frequency of the abortive states of line C; the high incidence of thenar/first interdigital patterns; the low incidence of hypothenar patterns; the frequency of the 5' level

of the axial triradii; and the abundance of whorls among the apical patterns) may be stated as definite racial characters. It is apparent, further, that with large groups tribal variations will be evidenced; such variations can only be suggested with numbers as small as these.

The trends in the palmar features noted above may be regarded as a racial accentuation of characters which in many peoples are regarded as sinistral traits. Since it has been shown in European-Americans that bimanual distinctions are correlated to a certain extent with lateral functional dominance, the finding in these Indians, the Mayas particularly, of a high percentage of left-eyedness, suggests that there may be also a high percentage of left-handedness or of ambidexterity.

- (29) 57. *A method of differentiating gonadotropic hypophyseal extracts from gonadotropic hormones of human pregnancy.* Samuel L. Leonard, Department of Anatomy, College of Physicians and Surgeons, Columbia University. (Introduced by P. E. Smith.)

It has been demonstrated that the ratio of the minimal ovulating dose of prolan (from urine of pregnant women) in rabbits to a minimal corpus luteum forming dose for immature female rats is approximately 2:1. This relationship does not hold for gonadotropic extracts of beef and sheep pituitaries where, in all cases examined, an inverse ratio of a considerable magnitude was revealed.

When this test was applied to human pregnancy blood serum, to an extract of human placenta and to an extract of the human hypophysis, it was found that the blood serum and placental extract behaved like prolan, giving a ratio of approximately 2:1. In contrast to this, the human hypophyseal extract gave a ratio of approximately 1:4, which strongly suggests a physiological difference between the gonadotropic hormones of pregnancy and the human pituitary extract.

This difference between the human hypophyseal preparation and prolan, placental extract, or pregnancy blood serum can be confirmed by another test. There is seemingly no definite limitation to the size of the immature ovaries produced by human hypophyseal extracts as is the case when the three products from human pregnancy mentioned above are used. It appears from these two tests that the human hypophyseal extract resembles the hypophyseal extracts of other species and is different from the gonadotropic products found in human pregnancy.

- (62) 58. *The essential nature of cells as inferred from their shape, with special reference to precartilag cells, and a surmise concerning the production of nerve cells.* Frederic T. Lewis, Department of Anatomy, Harvard Medical School.

Cell shapes show that cells result from the production of walls which under tension avoid 4-rayed intersections and tetrahedral angles. Cell division must be sharply distinguished from nuclear division. A binucleate cell is a syncytium. When the syncytium with two or more nuclei spaced at random is divided into cells by the production of walls with the properties just mentioned, the cells will be on the average 14-faced. Irregular as they may be, they will have

on the average thirty-six edges. They will vary in size according to their number of facets; and the division of the large ones, if occurring in a hexagonal plane, will not disturb this average. At the edges and corners, the wall common to two cells is under special tension and tends to split. The elastic double walls thus formed retract, making interstices, 'Zwickeln,' or gussets. Accumulation of substance primarily in these interstices leads to 14-rayed cells in a matrix; or, by breaking down or subdivision of the rays, to other forms than the primarily 14-radiate type. Expansion of certain cells in a 14-hedral aggregate, reversing the process which yields mesenchyma, appears to be the primary condition in the development of nerve cells. The probable manner of production of an aggregate of nerve cells and neuroglia from a 14-hedral medullary plate, will be sketched.

- (61) 59. *On the granular (coagulated) state of the nuclei of malignant rat sarcoma cells.* Warren H. Lewis, Department of Embryology, Carnegie Institution of Washington, and the Johns Hopkins Medical School.

The living malignant sarcoma cells so far examined in tissue cultures have granular nuclei in marked contrast to the clear homogeneous nuclei of normal living fibroblasts. This granular condition is more marked in the nuclei of some sarcomas than in others. Such malignant cells also have a heavier nuclear membrane than normal fibroblasts. The condition of these nuclei is similar to the reversible coagulation of the nuclei of normal fibroblasts produced by the addition of certain acids to the culture which can be reversed when the culture is again supplied with a normal medium. The similarity of the malignant nuclei to the coagulated state of normal nuclei is emphasized by the fact that when cultures of malignant cells were subjected to ammonia vapor or to ammonia buffers the granular condition of their nuclei disappeared, the nucleoplasm became homogeneous, and the heavy nuclear membrane became thin and scarcely perceptible. If such preparations were returned to a slightly acid medium, the granular condition and the heavy nuclear membrane returned. A more alkaline medium was needed to peptize the coagulated sarcoma nuclei than to peptize the coagulated normal ones and, conversely, a less acid medium to coagulate the peptized sarcoma ones than to coagulate normal nuclei. This leads to the assumption that the granular state of the nuclei of malignant sarcoma cells is a reversible coagulated state.

- (38) 60. *Comparison of threshold stimuli in nerve cilia and nerve muscle preparations.* Alfred M. Lucas, Washington University Medical School, St. Louis. (Introduced by E. V. Cowdry.)

The cilia in the roof of the frog's pharynx vibrate only when stimulated. Powdered charcoal, carmine, Ringer's fluid, blasts of air, and prodding with instruments are external agents which stimulate the cilia to activity from their quiescent state. These cilia may be activated also by impulses from the nervous system, either reflexly by stimuli to the tongue or directly by stimulation of the palatine nerve. The palatine nerve contains myelinated fibers 2 to 9 μ in diameter and small non-myelinated fibers. Results thus far using induced make and break shocks at the rate of 3 to 4 per second have repeatedly shown that a current

15 to 20 times greater is required to activate the cilia through the palatine nerve than is required to produce muscle contraction in the foot by stimulation of the peroneus nerve at the level of the tibiale and fibulare bones. The difference in threshold suggests that the autonomic fibers cause the cilia to vibrate. These results confirm evidence presented by McDonald, Leisure, and Lenneman ('28). No evidence of nerves inhibiting or slowing the movement of the cilia has as yet been obtained.

- (14) 61. *The corticifugal pathways for mastication, lapping, and other motor functions in the cat.* H. W. Magoun, Institute of Neurology, Northwestern University Medical School.

In order to reinvestigate the evidence pointing to the substantia nigra as an intercalated center in the corticifugal mastication pathway, the interior of the brain of cats was electrically stimulated with a bipolar needle electrode, oriented by means of the Horsley-Clarke apparatus.

In normal cats, rhythmic mastication, usually elicited as lapping, was followed back from the cortex in the internal capsule and basis pedunculi to the extreme caudal end of the pons.

In cats in which the anterior third of one cerebral hemisphere was removed and the brain stem stimulated after 6 weeks, the substantia nigra and the cerebral peduncle were negative on the operated side, while on the unoperated side rhythmic mastication was followed back in the basis pedunculi as in normal cats.

Incidental information on other motor functions points to the importance of the buried cortex lateral and dorsal to the presylvian sulcus, as a motor area, and indicates a medio-lateral distribution of head, fore limb, and hind limb in the responsive part of the internal capsule and rostral basis pedunculi.

- (76) 62. *The forces exerted on the human mandible by the muscles of occlusion.* Donald Mainland and J. Earle Hiltz, Department of Anatomy, Dalhousie University.

The object was to determine directions, relative amounts, and, approximately, absolute amounts (possible variations rather than averages) of forces of: masseter (superficial and deep), internal pterygoid, and temporal (anterior and posterior parts). Both sides of twelve heads of dissecting room subjects were examined, aged 22 to 87; five females, seven males (the temporal was measured on only three specimens). The methods were: determination of cross-sectional area perpendicular to all the muscle fibers, by inking cut surfaces, imprinting on paper, tracing on kodaloid and weighing; estimation of absolute force on a basis of 10 kg. per square centimeter; resolution of forces into components upward, forward or backward, outward or inward. The total forces (both sides) in three heads were: 308, 273, and 283 kg. Upward forces at second molars were comparable with gnathodynamometer results on living persons. The forward component there exceeded the upward. Possible ultimate combinations of forces were not analyzed, but tables were prepared to permit this. Certain results agreed with clinical opinion, e.g., greater average force associated with better molar dentition; a tendency for right muscles to be stronger than left. The direction (outward or inward) of resultant transverse forces depended largely on skull

shape. In most specimens the angles between muscles and eye-ear plane were in size-order: superficial masseter (least); pterygoid; deep masseter; but this was not always true.

- (24) 63. *The alteration of the characteristic effects of oestrin by extracts of corpus luteum containing progesterin.* Roland K. Meyer and Willard M. Allen, School of Medicine and Dentistry, University of Rochester.

Several investigators have demonstrated the presence in the pregnant animal of some mechanism whereby the characteristic effects of oestrin are altered, and some have suggested that the corpus luteum may be responsible for this alteration. In support of this concept, we have found that the simultaneous injection of oestrin and of purified corpus luteum extracts which contain progesterin, but only traces of oestrin, do not produce the oestrous condition in castrated mice.

These experiments were conducted as follows: Castrated mice were brought into oestrus and some were then injected for 8 days with six rabbit units of progesterin and six rat units of oestrin, and others with three rabbit units and three rat units. Control animals were injected with a total of either 3 or 6 rat units of oestrin.

The vaginal smears of the animals injected with oestrin and corpus luteum extracts consisted of leucocytes and a few epithelial cells and sections of the vaginae obtained at necropsy on the ninth day demonstrated the epithelium to be either of the mucous or transitional type, whereas the vaginal smears of those injected with oestrin only, consisted of cornified cells and a few leucocytes, and sections of these vaginae showed the epithelium to be cornified.

At the present time we are not prepared to state whether or not the factor responsible for these results is progesterin.

- (46) 64. *The effect of a new substance, 'prospermin,' occurring in normal male urine, on the gonads of the immature male rat.* J. A. Morrell, E. R. Squibb & Sons, New Brunswick. (Introduced by E. T. Engle.)

By processing normal male urine, using a method similar to that of Zondek and of Dodds, a substance was obtained which has certain specific effects on the male gonads. In immature male rats, 21 days of age, the daily administration of this extract for 7 to 10 days by itself produces some evidences of sex stimulation, as shown by increased development in the testicles and to a lesser extent by the secretory functions of the seminal vesicles and prostate.

The simultaneous administration of a growth preparation of beef pituitaries made by the Van Dyke method and prospermin for 7 to 10 days produces precocious spermatogenesis in such animals. They show completely formed spermatozoa at 31 days of age. The simultaneous administration of this material, prospermin, with pituitary hebin results in the precocious development of spermatogenesis.

The cortical hormone supplied by Doctors Swingle and Pfiffner by itself or in combination with the prospermin did not produce precocious spermatogenesis.

Pregnancy urine extract ('follutein') given by itself or in combination with growth hormone or pituitary hebin caused only an enormous hypertrophy of the

seminal vesicles and prostate with an increase in the amount of interstitial tissue, but no evidence of precocious spermatogenesis. Pregnancy urine extracts plus the cortical hormone show the same picture described above.

The age of normal maturity with the development of complete spermatozoa in these animals varies from 60 to 70 days.

- (34) 65. *The cerebrospinal fluid and the cervical lymph nodes in the living dog.* O. A. Mortensen and W. E. Sullivan, University of Wisconsin.

Under nembutal anesthesia, cisternal punctures were made in dogs and 2 to 4 cc. of cerebrospinal fluid was replaced by an equal amount of a radioopaque agent. The two substances used were brominol (light) and thorotrast (thorium dioxide). Roentgenograms of the head and neck regions were made immediately after the operation and at varying intervals up to 50 days, post operative. In general, the films reveal an almost constant filling of cervical lymph nodes, although the time of appearance and the extent of filling are variable. In the early experiments brominol was used and the time of appearance varied from 5 to 52 hours. Both superficial and deep nodes were frequently filled. When thorotrast was employed, the filling was more rapid, more complete, and lymph vessels as well as nodes are clearly demonstrated. Thorotrast can be recognized as early as 30 minutes, post operative, and after 1 hour a really good filling of nodes and vessels is apparent. Subsequent films up to 50 days show relatively little change in the distribution of the contrast media. The main route to the cervical nodes appears to be by way of the perineural spaces of the olfactory nerves into the lymph vessels draining the nasal mucous membrane. This interpretation in the living animal was confirmed by gelatin injections and is in agreement with previous workers. (See demonstration no. 26.)

- (89) 66. *The fetal membranes of the Sciuridae and their significance.* H. W. Mossman, Department of Anatomy, University of Wisconsin.

The squirrels exhibit a peculiar type of implantation which might be regarded as highly specialized. However, their fetal membranes as a whole seem to represent a primitive rodent type. This view is supported by these facts: they have a very well developed true yolk-sac placenta, followed by incomplete inversion of germ layers; a tardily developing simple type of labyrinthine hemochorial allantoic placenta with no specialized areas and no tendency to reduce the trophoblast of the placental membrane as in most other rodents; a small amount of decidua composed of cells not as highly modified as is usual; and only 1 or 2 types of giant cells. They demonstrate beyond much doubt the origin of the familiar giant cells of the uterine wall from the trophoblast. Reichert's membrane projects into short, inwardly directed endodermal villi of the bilaminar omphalopleure, even where ectoderm is absent, thus indicating that it is formed by the endoderm cells. We have been impressed while studying these rodents with the advisability of discarding the loose use of the term 'yolk-sac placenta' in favor of some terminology which will distinguish between the true yolk-sac placenta (chorion vascularized by yolk-sac vessels), the bilaminar omphalopleure, and the inverted vascular splanchnopleuric wall of the yolk-sac most highly developed in rodents with complete and early inversion of germ layers. (See also abstracts nos. 154 and 156, demonstration no. 27.)

- (50) 67. *The prevention of castration changes in the rat hypophysis by the administration of oestrin and of testis hormone.* Warren O. Nelson, Hull Zoölogical Laboratory, University of Chicago. (Introduced by Carl R. Moore.)

Approximately 220 male and female rats have been subjected to gonadectomy and subsequent treatment with either oestrin or testis hormone in graduated amounts for periods of time, ranging from 10 to 30 days. These animals were sacrificed and their hypophyses removed, weighed, and fixed for histological examination. For control purposes hypophyses from over 100 normal and untreated castrates have been studied.

Oestrin injected in amounts as low as 1 rat unit daily prevented the cell changes of castration in the female hypophysis over a 30-day period. One-half rat unit was not completely effective. Male animals required 4 to 5 R. U. daily for the same duration of castration.

Testis hormone (Koch-Gallagher preparation) administered in dosages as low as 3 bird units daily regularly prevented castration changes in female hypophyses. Experiments with lower doses are in progress. Amounts progressively increasing with the duration of castration have been necessary for the prevention of castration changes in the male. In 11-day castrates 6 B. U. have been effective, while in 21-day castrates 7.5 B. U. maintained a normal hypophysis, but failed to do so in 30-day castrates. Higher doses are being administered at the present time.

This study demonstrates that either oestrin or testis hormone is effective in suppressing, in either sex, the cell changes which occur following gonadectomy in the rat, but that a much higher concentration of either hormone is required in the male than in the female. (See also abstract no. 157.)

- (67) 68. *The development of rat embryonic tissues after transplantation of the egg to the kidney.* J. S. Nicholas, Osborn Zoölogical Laboratory, Yale University.

The present experiments include transplantations of segmenting eggs, blastulae, blastocysts, and early embryos contained within their membranes. These were placed under the capsule of the adult kidney. This location, the efficacy of which was discovered by Adrian Buyse, of the University of Rochester, has distinct advantages over those previously described.

The capsule holds the transplant in place; it is easy to differentiate in the gross between the tissues of the host and the graft; the percentage of successful differentiating grafts is equal to that secured in the mammary gland. The kidney capsule does not form an investing connective tissue layer completely enclosing the embryo, and on this account this region is excellent for maintenance of grafts.

When 2-celled eggs are transplanted to this location, tissues arising from each of the three germ layers are found in the grafts recovered after 14 days. Cartilage, bone, gut, nerve elements, skin, and muscle, both striated and smooth, are completely although irregularly differentiated. The embryonic membranes are absent in such grafts, although they were well formed in the older stages at the time of transplantation.

- (23) 69. *Will prolonged injections of ovarian or pituitary hormones combined with chronic trauma produce a precancerous condition in the cervix of the monkey?* Milton D. Overholser and Edgar Allen, Department of Anatomy, University of Missouri.

Cervical trauma was produced by scissor cuts at weekly intervals. In addition, a metal clip was placed in the cervix of some animals.

Injections of hormones were made twice daily. Five spayed animals were given theelin or amniotin, ranging in total dosage from 1535 to 4747.5 rat units, over time periods ranging from 16 to 90 days. Two animals also received corpus luteum hormone (corporin or progestin) during the latter part of the theelin injections. Two semispayed animals were given follutein (anterior pituitary sex hormone from urine of pregnancy), 990 units in 37 days and 6500 units in 142 days, respectively.

A spayed control received cervical trauma only (86 days). A normal control receiving trauma alone is still living.

In all experimental animals atypical epithelial hyperplasia and possibly metaplasia occurred at the junction of the stratified squamous and glandular epithelium. In many regions columnar epithelium was surrounded by stratified squamous. Downgrowth and irregularity of the rete malpighii occurred with formation of isolated epithelial masses. In some cases nuclear changes were seen and a basement membrane was lacking. Similar conditions occur in human material about old cervical tears and erosions. It seems possible that a precancerous condition has been experimentally produced in these animals.

The spayed control (trauma only) was negative. The normal control and two additional experimental animals are still to be studied.

- (59) 70. *The races that constitute the group of common fibroblasts.* Raymond C. Parker, Rockefeller Institute for Medical Research. (Introduced by R. G. Harrison.)

Common connective tissue cells, or fibroblasts, have always been assumed to belong to a single cell type. Their morphology is well known. After they had been obtained in pure cultures, they could be identified not only by their appearance, but also by their functional properties.

Recent experiments have shown that fibroblasts, as a group, comprise many different cell types. When, for example, 10 to 20 cell strains are isolated simultaneously from a chick embryo, and cultivated for long periods under identical conditions, it is found that they differ not only in their rate of multiplication in a given environment, but also in the amount of acid they secrete, in their ability to digest fibrin, etc. Furthermore, these characteristics remain constant.

Cell races showing pronounced nutritional differences, equally persistent, may also be isolated from corresponding parts of embryos of different ages. Thus, fibroblasts derived from a 20-day-old embryo may show, under the same conditions, a rate of multiplication that is either higher or lower than that exhibited by similar strains obtained from a 10-day-old embryo, depending upon the particular organs or tissues selected. The physiological properties of fibroblasts depend solely upon their origin. In fact, no two races of fibroblasts can be

relied upon to react identically to the same surroundings, even though removed simultaneously from exactly the same part of two individuals of the same age and species.

- (43) 71. *The beginning of peristaltic contraction in the embryonic chick heart.*
Bradley M. Patten and Theodore C. Kramer, Western Reserve University.

The present communication covers the continuation of the work on early embryonic heart action presented at the 1932 meetings. Micromoving pictures of living hearts have been analyzed by the method of superimposed tracing of enlargements from successive frames. This method shows that there is: 1) a coordination of the first fibrillar contractions into contractions involving the entire ventricular myocardium prior to the beginning of a peristaltic type of action; 2) that peristaltic action begins in the heart at the time when the previously paired atrial primordia are incorporated in the unpaired cardiac tube posterior to the already formed and beating ventricle; 3) that the peristaltic character of the beats becomes more marked with the entrance of blood into the heart which adds the factor of passive dilation of the heart and therewith stretching of the muscle just prior to its contraction phase. Evidence is presented from previous or current work, showing that the intrinsic contraction rate of the primitive atrium is more rapid than that of the ventricle. The possible bearing of this fact on the beginning of a peristaltic type of contraction and on the rate of contraction of the heart as a whole is discussed. (See motion picture demonstration no. 2.)

- (12) 72. *Observations on the structure of the vagus nerve.* S. W. Ranson, J. O. Foley, and C. D. Alpert, Institute of Neurology, Northwestern University Medical School.

In the cat's vagus motor and sensory fascicles can be followed from the periphery of the medulla as far as the nodose ganglion. At the cephalic pole of this ganglion the motor fascicles are often entirely separate from the main trunk and give to this part of the vagus its characteristic fasciculation. After intracranial section of the vago-accessory rootlets the motor fascicles degenerate while the sensory ones remain intact.

The fine unmyelinated fibers in the sensory fascicles persist after intracranial section of the roots and after removal of the superior cervical ganglion. They represent the fine central branches of axons of nodose ganglion cells and are much more delicate than the sensory unmyelinated fibers below the level of that ganglion which represent the peripheral branches of the same axons.

The nodose ganglion contains, in addition to simple unipolar neurones, many fenestrated cells and some with appendices terminating in end bulbs, but no cells with multiple long dendrites like those seen in sympathetic ganglia. It contains no intercellular plexus of fine branching preganglionic fibers. There is no histological evidence that it contains synapses between pre- and postganglionic parasympathetic neurones. (See demonstration no. 30.)

- (48) 73. *The relative number of the different types of cells in the adult human female hypophysis.* A. T. Rasmussen, Department of Anatomy, University of Minnesota.

This report is based on a differential count of the cells in the anterior lobe of ninety-four carefully selected normal non-pregnant female hypophyses (16 to 84 years of age) from cases of sudden accidental death and on twenty-five pregnant women (15 to 39 years of age). Total cells counted in each hypophysis averaged 15,000, representing all the cells containing nuclei in 214 (on an average) equally spaced microscopic fields (oil immersion lens) from each hypophysis. (Section 5 μ , Mallory's acid fuchsin-orange G-aniline blue stain after slight preliminary staining with hematoxylin.)

In non-pregnant women the chromophobes average 49.6 per cent, coefficient of variation, 19; acidophils average 43.4 per cent, coefficient of variation, 19; basophils average 7 per cent, coefficient of variation, 42. In those over 50 years of age there are 6 per cent less acidophils and correspondingly more chromophobes and basophils (4 per cent and 2 per cent, respectively). There is no correlation between the percentage of any type of cell and stature.

There is a slight (2 per cent), but not significant, increase in the percentage of chromophobes and corresponding decrease in the percentage of acidophils during pregnancy. The anterior lobe of the hypophysis enlarges during pregnancy, but no special so-called pregnancy cell could be identified.

Women, in addition to having an absolutely larger anterior lobe, have relatively more (about 7 per cent) acidophils, and hence a smaller percentage of chromophobes and basophils, than men.

- (94) 74. *Changes in the venous system incident to changes in relative position of the embryonic heart; our fundamental conception of the term 'cardinal vein.'* Franklin P. Reagan, Department of Anatomy, College of Medicine, University of Illinois.

Ventro-laterally (morphologically ventro-medially) to the uppermost portion of the primitive posterior cardinal line of the amniote embryo is a plexus which I have previously described and have called the plexus triangularis. That portion of the primitive duct of Cuvier which borders this triangular plexus later becomes transformed into a part of the anterior cardinal vein of mid-embryonic life. From the lower border of the plexus is resolved the upper portion of the posterior cardinal veins of mid-embryonic life. That portion of the primitive posterior cardinal line which dorsally borders the triangular plexus disappears. This latter portion of primitive posterior cardinal line was first designated by Sabin as common 'cardinal vein.' As a corruption of that terminology, the duct of Cuvier of mid-embryonic life has been called common 'cardinal vein' by subsequent authors. In any case, the term 'common cardinal vein' is a misnomer, and in no event should replace the term ductus Cuvieri of Rathke (1836). It was applied by Sabin to a vessel which forms no part of ductus Cuvieri.

Additional facts concerning the behavior of the internal mammary vessels will be considered along with general problems of change of relative position of the heart.

- (93) 75. *The origin of the azygos and hemiazygos veins in man.* George A. Seib, Department of Anatomy, Washington University School of Medicine. (Introduced by R. J. Terry.)

The entire azygos system of veins was dissected in 200 cadavers (100 American whites; 100 American negroes). It was found that the azygos and hemiazygos veins had their origin in the thorax from 1, 2, or 3 roots: a lateral root, bilaterally present in 85 per cent; an intermediate, present on the right side in 45 per cent; on the left, in 28 per cent; and a medial root, present on either side in about 40 per cent of cases.

The lateral root was a common trunk formed by the union of the ascending lumbar and subcostal veins. The intermediate root was usually formed by a vein which arose on the right side from the inferior vena cava (the lumbar azygos vein) and on the left side from the left renal vein (the lumbar hemiazygos vein). The lumbar azygos and lumbar hemiazygos veins then continued craniad, traversed the diaphragm via the crura, and terminated by continuing directly into the respective azygos and hemiazygos veins. The medial root of origin was formed by a vein (subcentral vein) which usually arose from the inferior vena cava, sometimes the left renal, and passed craniad, dorsal to the aorta, to enter the thorax. The subcentral vein then divided, usually, into right and left branches (right and left subcentral veins) which emptied into the beginning of the azygos and hemiazygos veins, respectively.

- (54) 76. *Cytological observations on the thyroid activated by anterior pituitary.* Aura Edward Severinghaus, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Cytological studies on the activated thyroid of the duct ('31, in collaboration with Doctor Schockaert) have been extended to include the guinea pig, sheep, and monkey. Aside from the cellular hypertrophy, hyperplasia, the constant enlargement and apical position of the Golgi apparatus, and colloid emptying of the follicles, certain more specific features are of interest.

Degenerating cells (colloid cells of Langendorff) are more numerous in the thyroid of the duck when activated, and frequently afford entry for capillaries into the follicular lumen. In early stages of stimulation low dark staining cells make up one side of the follicle, the opposite side having tall clear cells. Cells in the walls of abutting follicles are of opposite type.

In the sheep, following colloid depletion, the follicular lumen is often solidly filled with proliferating cells which lose their characteristic shape, size, and polarity. Certain cells greatly distend with newly formed colloid and become centers about which other cells polarize (as seen by the Golgi positions) to form new follicles within the original one.

The most striking feature in the activated thyroid of the monkey is the abundance of cells showing basal colloid vacuoles similar in position, but much more distinct than those described by Bensley. Apical vacuoles of similar character, as well as colloid droplets which stain more deeply, are also found. The Golgi is invariably apical.

- (11) 77. *The development of an accessory abducens nucleus in the pig.* R. F. Shaner, University of Alberta.

In 16-mm. pig embryos, in which the great migration of the facial nucleus is going on, a lesser migration from the abducens also takes place. A small group of cells from the lateral part of the abducens nucleus moves ventrolaterally and comes to lie in 20-mm. embryos just rostral to the facial nucleus, in the plane of the superior olive, and close to the lateral fillet. Axones from the accessory nucleus run back over the path of migration in loose wavy strands to join the nerve rootlets just below the main nucleus. The accessory abducens nucleus is very conspicuous in Cajal preparations of embryos up to 100 mm. and can be identified in the adult brain.

- (10) 78. *Studies of the mesencephalic nucleus in the normal and experimental cat.* John J. Sheinin, Department of Anatomy, Northwestern University Medical School.

In addition to usual type A cells, two new types (C and D) were found. Intracranial section of either the trigeminal or mandibular nerve caused complete chromatolysis of A and C cells on the operated side. Type D cells normally resemble chromatolyzed cells; they comprised only 2 to 5 per cent of the total number. Unilateral removal of the orbital contents caused considerable chromatolysis in the homolateral mesencephalic nucleus, accompanied by marked chromatolysis in the third and fourth motor nuclei in all animals. No chromatolysis appeared in the fifth motor nucleus in two animals, and only a small amount in one animal. Hence chromatolysis in the mesencephalic nucleus of the fifth nerve was caused by cutting the mandibular or by removing the extra-ocular muscles without damaging the muscles of mastication. This indicates multipolarity of the mesencephalic sensory cells (or bifurcation of their processes) and a distribution of processes of the same cell to the extra-ocular muscles as well as to the muscles of mastication. Examination of silver preparations showed that many of the cells send more than one process (which may bifurcate) into the mesencephalic tract. Distribution of two or more processes from the same cell to different peripheral nerves would account for much of the earlier lack of harmony in the findings of different investigators as well as the apparent paradox in the present results.

- (92) 79. *Studies on the human omentum, with special reference to its lymphatics.* Parke H. Simer, Department of Anatomy, College of Medicine, University of Illinois. (Introduced by Otto F. Kampmeier.)

Histological study of fetal and adult omenta shows the presence of lymph vessels with functional valves from the beginning of the fourth fetal month. These vessels persist throughout fetal life and do not degenerate, as has been claimed by some investigators. They are profuse in the adult omentum and are found even in the distal end of the omental sac; that is, in those portions furthest removed from its attachments. The lymph vessels form a characteristic plexus around the arterial highways and communicate with other more solitary channels that drain adjacent territories. Valves occur in the lymph vessels at intervals of from 1 to 2 mm. Valves are present also in the veins, but are much less frequent. Nerves accompany the vascular channels throughout the entire omentum.

- (87) 80. *A study of the morphological physiology of the frog's kidney with the fluorescence microscope.* Edward Singer, Department of Anatomy, College of Physicians and Surgeons, Columbia University. (Introduced by S. R. Detwiler.)

A microscopical study of the living kidney in situ was made with the aid of reflected white light and fluorescent light.

The main source of the fluorescent illumination was a concentrated solution of aesculine which appeared colorless in the white light. Thus we had a stained tissue in fluorescent light and an unstained tissue in white light; consequently, a constantly interchanging observation of the stained and unstained tissue was possible. Fluorescent tissue staining in both lights was obtained by the use of neutral acriflavine.

An intermittent circulation was observed only in damaged glomeruli.

An uneven distribution of injected substances in the large veins was noted.

The elimination of aesculine and acriflavine was observed on the basis of the sequence in which their fluorescence was visible in the different portions of the uriniferous tubule.

The evidence shows that there is a resorption of substances in the proximal convoluted tubules. This does not exclude the possibility of a secretion of substances by these parts.

The flow of urine through the proximal and distal convoluted tubules is intermittent. By filtration through the capsule the proximal convoluted tubule gradually fills, the urine being retained here for a short period. This tubule then empties, the urine flowing rapidly through the connecting and distal tubules to the collecting duct.

- (28) 81. *Genital responses of hypophysectomized female rats to pregnancy-urine injections.* Philip E. Smith and Samuel L. Leonard, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Pregnancy-urine extracts have been injected in immature and mature hypophysectomized female rats. The treatments were begun from 0 to 54 days after operation. The duration of treatment varied from 3 to 34 days. Control ovaries were secured by unilateral ovariectomies and from untreated hypophysectomized litter mates. Changes following cessation of treatment were also studied.

A constant and pronounced enlargement of the ovaries has been secured, except in prolonged treatments after the longer periods of hypophysectomy. The actual size of the ovaries, when injections are begun immediately after operation, is greater than when treatment is postponed, although the relative increase may not be as great. The enlargement is not due to follicular growth, but to a hypertrophy of the cells of atretic follicles and of the existing cords of interstitial tissue. Except for their arrangement, these cells, although somewhat smaller, appear similar to true lutein cells. With cessation of treatment rapid regression occurs. Some of the transformed atretic follicles persist as discrete bodies, similar to small corpora lutea.

An oestrous (cornified) smear appears early and continues except for an occasional mixed type, when treatment is begun immediately. A dioestrous type predominates when the beginning of treatment is postponed. The weights of the uterus are maintained or increased in animals treated immediately after hypophysectomy and is increased in those in which atrophy has occurred.

- (57) 82. *The development and minute structure of certain hereditary tumors in Drosophila.* Mary B. Stark, New York Homeopathic Medical College and Flower Hospital.

This report will concern two tumors: one a lethal occurring in one-half of the males and killing same; another, appearing in both males and females and always benign.

The sex-linked tumor occurring at any time during the larval life, always interfering with further metamorphosis and eventually killing the larva, is derived from imaginal discs, groups of tiny embryonic cells, destined to form some of the internal organs of the fly. Those of the digestive tract of the larva have been found to enlarge by rapid proliferation of their cells and break away from their original positions, developing into structures one-fourth as large as the larva itself. The cells are unequal in size, and become filled with a pigment.

The benign tumor, which is due to genes located in the second, third, and possibly the fourth chromosomes, takes its origin in dorsal segmental organs located below and on each side of the dorsal aorta in the larva stage. The tumor is carried into the adult fly in which it becomes completely encapsulated and seems apparently harmless to the fly.

- (6) 83. *Genetic constitution and endocrine environment in structural expression.* C. R. Stockard, Cornell University Medical College, New York City.

In various crosses between structurally contrasted pure breeds of dogs localized reactions occur indicating effects of one or more genes in determining a given structural pattern in one body region and a modified pattern in another region, in spite of the common chemical or endocrine environment of the several regions. A significant example of these facts is shown in the inheritance of the short-twisted tail of the English bulldog when crossed with the long-tailed short-legged basset hound. The bulldog tail is a double recessive in inheritance and related to two factors probably located in different chromosomes. The homozygous double recessive appears in the second generation in only one in sixteen individuals.

Chondrodystrophy of the skull and tail vertebrae in the bulldog may be thought of as similar reactions to a common internal cause. However, second-generation hybrid individuals may show almost normal hound-like heads and yet possess the deformed short-twisted tail. Such individuals, homozygous for both recessive factors determining 'screw-tail,' develop chondrodystrophy of the tail in the same endocrine environment in which the skull and extremities are developed quite normally. This indicates that general endocrine modifications are ineffective in regulating the type of growth response in opposition to the genetic constitution. For illustration, ordinary individuals may not respond alike to pituitary modification with those inheriting acromegaly.

- (69) 84. *A detailed study of developing lateral-line sense organs in embryos, larvae, and adults of Amphibia based on living and vital-stained specimens.* L. S. Stone, Department of Anatomy, Yale University School of Medicine.

The development of lateral-line sense organs in living embryos, larvae, and adult Amphibia was studied in detail under high magnification with the aid of specially devised observation chambers. Selectively stained migrating lateral-line

primordia from grafts dyed with Nile-blue sulphate separate into blue bead-like segments. These are deposited as the sole origin of the sensory and supporting cells of the primary lateral-line organs. By observing the streaming activity of dye in the cells, it was possible to follow the organ formation from early stages to the fully developed structure.

In larvae the organs increase in number by budding from the supporting cells at definite poles of the primordial organs. This was clearly demonstrated by following the dye-laden cells in the formation of the normal linear groups of accessory organs and by inducing budding in organs at definite poles directed toward an area of rapid proliferation in regenerating tails. The same process of budding is called forth in the development of new organs in regenerating tails of adult salamanders. The essential features of development are the same in embryos and larvae of anurans as in similar stages and adults of urodeles. Observations on living specimens, in which the dye could be observed for several weeks, were correlated with those obtained from special histological preparations in which the dye was perfectly preserved.

- (7) 85. *Primate embryos made to order.* George L. Streeter, Department of Embryology, Carnegie Institution of Washington.

Because of the light they throw on age of human embryos, the author will show a series of macaque embryos of known ages which were obtained from Doctor Hartman's colony. The rate of growth and differentiation in man and monkey is assumed to run closely parallel in the early part of development, and during this period the progress in differentiation appears to be sufficiently rapid to offset variations between individuals. Checked in this way, the Peter's embryo is rated at 13 days, fertilization age. At 20 days, Hensen's node, a notochordal process and primitive groove are present. The first somites make their appearance on the twenty-second day. Segmentation of somites proceeds rapidly as 1, 5, and 8 somites are found in same day. A 6.5-mm. embryo with triangular fin-like arms and beginning leg buds was obtained on the twenty-ninth day. Older specimens were obtained as follows: 32 days, 10.5 mm.; 35 days, 12 mm., with definite finger rays; 38 days, 13 mm.; and 75 days, 110 mm.

- (32) 86. *Development of the tela chorioidea in the albino rat.* R. M. Strong and Charles M. Jessico, Department of Anatomy, Loyola University School of Medicine.

This paper reports a study of the development of the tela chorioidea and choroid plexus in the albino rat. The research is a part of a program of studies of the meninges of the albino rat and human brain.

In general, our observations agree with those of Weed on this subject, using pig embryos. Stages beginning with 11 days prenatal have been studied, and will be described with the aid of lantern slides.

- (22) 87. *Uterine bleeding after section of the spinal cord.* G. van Wageningen and M. Kennard, Yale University School of Medicine.

Transection of the spinal cord of monkeys at levels varying in different animals from the eighth thoracic to the twelfth have been followed by uterine bleeding

in seven cases, five macaques (*Pithecus rhesus*) and two mangabeys (*Acrocebus aethiops*). The bleeding in each instance came well before the expected menstruation, but otherwise was similar in duration and character. There was an average interval of $5\frac{1}{2}$ days after the operation before the appearance of blood in the vagina. A control animal which had been oophorectomized did not react in this way. As postoperative care was improved, it became possible to keep three animals in good physical condition for periods of several months, and in these menstrual cycles of normal duration became reestablished. One animal had postoperative cycles of 24, 25, and 29 days despite profound atrophy accompanying the paralysis of the body caudal to the transection. In this animal the ovaries and uterus were small, yet autopsy on the third day of the last menstruation showed an actively bleeding uterus. In all cases the transections were made early in the cycle, presumably before ovulation. The histological preparations of the uterine bleeding following operation resembled those of normal menstruation from interval endometrium.

- (55) 88. *The enlarged thymus as associated with tubular cysts.* E. M. Vicari, Department of Anatomy, Cornell University Medical College, New York City. (Introduced by C. R. Stockard.)

Sudden deaths among puppies of 1 to 3 months of age were found upon autopsy to be associated with the presence of enlarged thymuses. The similarity of these cases to the so-called thymic deaths of children is suggestive and may throw light on the nature of this occurrence. The cause of thymic enlargement and its significance is little known and few satisfactory studies have been made on children. The enlarged thymuses observed in puppies (twelve of them) were either of a solid compact type or a gelatinous, oedematous type. Microscopic study disclosed epithelial tubular cysts connecting additional thymic lobules with the thyroids and parathyroid glands and thyroid follicles, on the other hand, were found enclosed within the thymus mass. The tubular cysts may be remnants of epithelial stalks originally connecting the thymus with the pharyngeal pouches and their persistence is evidence of an arrested developmental reaction in the thymic formation and possibly in the branchial region generally, since they are more or less fused and mixed with the other branchial glands. In puppies these tubular cysts have been observed chiefly in the German shepherd-basset hound hybrids and in fewer cases in the basset hound-British bulldog hybrids.

- (58) 89. *Relative distribution of the cells in the peritoneal fluid of the white rat, between birth and sexual maturity.* Richard L. Webb, Department of Anatomy, College of Medicine, University of Illinois.

Between birth and sexual maturity, marked changes appear in the relative distribution of the cells within the peritoneal fluid of the white rat. During the entire period, the basophils maintain the relative percentage (approximately 7 per cent) which is normal for the adult. The eosinophils form a minor part of the total number of cells (approximately 0.6 per cent) of the newborn, but they gradually increase until the percentage found in the normal adult is attained at sexual maturity. The group of mononuclear cells composes approximately 90 per cent of the cells in the peritoneal fluid of the newborn. They

gradually decrease until they reach the level of approximately 62 per cent at the time of sexual maturity. The neutrophils are absent during the entire period.

- (15) 90. *Correlation between the development of local reflexes and unisegmental reflex arcs in the spinal cord of cat embryos.* William F. Windle, Anatomical Laboratory, Northwestern University Medical School.

The first somatic motor response to adequate light pressure stimulation occurs in the fore limbs of 13 to 14 mm. embryos. It is a quick local flexion, usually following an appreciable latent period, and often, apparently, requiring summation of stimuli. In these respects, it differs from movement resulting from direct faradization of embryonic muscle. Unit fore limb reflexes precede and appear not to individuate from the massive pattern of trunk motility in the cat.

All elements of primitive reflex arcs are present in the spinal cords of premitile embryos. However, the sensory fibers of the posterior funiculus lack or are just beginning to develop collaterals to connect with association neurones. Sensory collaterals were enumerated in representative spinal segments in a series of motile and nonmotile specimens of similar ages and sizes. About two and one-half times as many occur in motile embryos as in the nonmotile ones; the greatest increase is in the brachial region. The first collaterals appear at the entrance points of the dorsal roots, impinging upon association neurones at the same level. The latter can effect connections with ramifying dendrites of motor neurones of the same segment. This is clearly a mechanism for unisegmental reflexes. It is suggested that the physiological responses, so intimately coordinated with the development of the sensory collaterals, appear in consequence of their growth and are true unit reflexes.

- (30) 91. *The effect of x-ray sterilization and cryptorchism on the hypophyseal functions.* Emil Witschi and W. T. Levine, University of Iowa.

X-ray sterilized male rats maintain fully and similarly treated females eventually retain partly their secondary sex characters. When tested by parabiotic union with normal females, however, they effect similar changes as surgical castrates, i.e., they cause prolonged cycles or constant oestrus in the female twin. Cryptorchids give the same reactions as x-ray sterilized males. Obviously, the endocrine activity of the sterile gonad, even though sufficient to maintain secondary sex characters, does not prevent the hyperfunction of the hypophysis that is characteristic for castrates.

- (47) 92. *Cell types found in the anterior hypophyses of various species.* J. M. Wolfe and Rucker Cleveland, Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine.

The cells of type I (eosinophiles) of the anterior pituitaries of dogs, sows, rats, rabbits, guinea pigs, and humans are identical. These cells include small and large forms; the cytoplasm is colorless. The closely packed granules of the smaller cells stain intensely with orange G. The cell membrane is distinct. The granular content of the larger cells is reduced during the lutein phase. In

such instances, the granules stain lightly with orange G. The cell membrane may be indistinct. Two basophilic types were differentiated in the dog, types II and III. The granules of the former stain deep red, varying to orange-red. Blue cytoplasm is found in only the smaller cells. The granular content of the larger cells is often reduced (lutein phase). The granules of the cells of type III stain violet or dark blue. The granular content of these cells is reduced in oestrus. Blue cytoplasm is found in all forms of these cells. In other species studied, one basophilic type was differentiated (classified as type III). Except in rats, these cells exhibit characteristics of both the basophilic types of the dog. In rats (non-pregnant) the basophilic cells are similar to the type III cells of the dog. The cells of type IV (chromophobes) are similar in all species studied; in these cells the cytoplasm is either fragmentary or unstained. (See also abstract no. 188 and demonstration no. 39.)

- (56) 93. *Observations on the follicular cycle and the presence of 'macrothyrocytes' in the human thyroid.* Gustav Zechel, Department of Anatomy, College of Medicine, University of Illinois.

The follicular cycle and the large thyroid cells, pointed out by the writer earlier (1931) in the thyroid of the dog, also occur in the human thyroid. In man the conditions are, however, not so pronounced. In histological sections, the human thyroid shows areas of disintegrating follicles, and other areas of young and newly formed follicles. Here and there throughout the sections there is evidence also of undifferentiated thyroid tissue. Careful examination is necessary to find the large thyroid cells, or 'macrothyrocytes,' because this cell type does not occur as frequently as in the dog. Nonidez, in observations made independently (1932), confirmed the presence of such cells in this animal, and his descriptions and interpretation were similar to the account given by the writer. In a more recent paper, Nonidez expresses opinions which are at variance, but one cannot avoid seeing the contradictions in his statements.

- (91) 94. *On the development of the lymphatic system in opossum (Didelphys virginiana).* Arnold A. Zimmermann, Department of Anatomy, College of Medicine, University of Illinois.

The lymphatic system in opossum embryos appears between 6- and 6.9-mm. stages. The jugular lymph sacs and the thoracic duct develop simultaneously and independently of each other. Until birth (10.5 mm.) the jugular sacs predominate with two widely divergent cephalic and axillary extensions. In the 8.5-mm. embryo they join the venous system by a valve located medio-ventrally at the junction of external and internal jugular veins. The axillary portion is continuous with a subcutaneous sac which peripherally merges with an extensively degenerating venous plexus and its replacing mesenchymal vacuoles.

The thoracic segment of the thoracic duct arises first, apparently from mesenchymal spaces in the mediastinum ventral to the aorta, and soon forms a plexus which in an 8.5-mm. embryo reaches from diaphragm to level of junction of azygos and common cardinal veins. At this stage the plexiform duct is continuous with minute branches of the left azygos vein.

The cervical portion or arch of the thoracic duct develops last. In the 8.5-mm. embryo there are short medio-dorsal extensions of both jugular lymph sacs which terminate in mesenchymal vacuoles surrounding collapsing venules of the mediastinum at a level far above the cranial blind end of the thoracic segment of the duct.

No definite inguinal lymph sacs occur before birth. There is no evidence of anterior or posterior lymph hearts at any stage.

PAPERS TO BE PRESENTED BY TITLE

95. *On the gonad- and thyroid-stimulating potencies of phyone and hebin.*

A. Elizabeth Adams, Zoölogy Department, Mount Holyoke College.

Normal and hypophysectomized adult female *Triturus viridescens* were injected daily (0.2 cc. or 0.5 cc. dosage) with 1:10 solutions of phyone or hebin (Wallen-Lawrence and Van Dyke). Both extracts induced egg-laying (or ovulation) in normal females (8 of 10 tests made with each extract), the reaction occurring at an average of 9.6 days for phyone- and 10.1 days for hebin-injected animals. Evidences of molting were observed in 18 of 20 normal females given both treatments, the range of molts being 1 to 9 for the nine molting phyone-injected animals treated for 5 to 20 days and 1 to 7 for the nine molting hebin-injected ones treated for 7 to 20 days. Of nineteen hypophysectomized females injected with the extracts, fifteen molted; i.e., shed the cornified epidermis which accumulates as molting is inhibited after hypophysectomy. The remaining four died before any reaction. No significant difference appeared in the time at which molting was first observed (average of 74.1 hours for the seven molting phyone-injected animals; 73.3 for the eight molting hebin-injected ones). Thyroids of normal animals showed marked and similar stimulation with both extracts, as did those of molting hypophysectomized ones.

The data are interpreted as indicating both gonad- and thyroid-stimulating potencies in phyone and in hebin and as further evidence of a pituitary-thyroid control of molting in *Triturus*. (See demonstration no. 3.)

96. *A further study of the pars villosa of the lip in human infants.* Barry J.

Anson and Roland C. Wherry, Department of Anatomy, Northwestern University Medical School.

In an effort to bring to a close a comprehensive study of labial villi in vertebrates (reported at several earlier meetings), the writers have collected a considerable amount of new data bearing particularly upon the appearance of the so-called 'villous' zone in infants' lips. All of the data substantiate opinions already recorded, which are in part as follows: that the pars villosa of the infantile lip, although invariably recognizable as a zone, does not normally in the living possess villous outgrowths; that fixed preparations of lips regularly display at least low nodular projections, and that macerated ones show an astonishing array of tall free villi; that the papillary condition of the lip is therefore artificial, and due to shrinkage or to desquamation of the epithelium—a change which brings the underlying dermal papillae into greater or lesser prominence.

For the portion of the study here reported, and in addition to previous observations, the following material has been examined: lips of 110 living infants; thirty-four excised specimens, either fixed immediately or following planned maceration; from this group, thirteen were serially sectioned, and several modeled by the wax plate method to demonstrate the exact extent to which epithelial contraction or desquamation had occurred.

97. *On the annular and helical arrangement of muscle in the veins of the mammalian liver.* L. B. Arey and Robert Sohlberg, Jr., Northwestern University Medical School.

In a study involving the livers of twenty-nine species of wild and domestic mammals the regular occurrence of veins with an atypical tunica media was found in two only, the raccoon and the dog. These vessels have been studied in detail and reconstructed in wax.

In the raccoon, the central and sublobular veins, and even somewhat larger hepatic tributaries, have their smooth muscle content segregated in distinct rings spaced rather far apart. Only rarely, except at bifurcations, do branched rings occur. Large hepatic tributaries lose this annulation and contain scattered smooth muscle, circularly arranged. Similar but more delicate series of rings occur in the portal veins of portal canals.

In the dog, the sublobular veins bear smooth muscle arranged in a widely spaced helix. At intervals this fundamental spiral may be reinforced by complete rings. In larger hepatic tributaries the helix is incomplete and frequently interrupted. Imperfect rings, with a heavy massing of muscle on one side, are a characteristic of such vessels.

98. *The number of taste buds on human circumvallate papillae.* L. B. Arey and M. J. Tremaine, Northwestern University Medical School.

A study of 28,205 taste buds on 116 papillae and trench walls gives a mean of 196 to each papilla and 48 to each wall. The greatest and smallest numbers found were 788 and 5 for papillae and 231 and 0 for walls. The number of taste buds on papillae remains unchanged between birth and beginning old age, but there is apparently a reduction in advancing old age. Certain other data indicate that the full number of taste buds on the trench walls is not attained until after the first year of life. Eliminating individuals over 70 years, the means become 208 for the papilla and 48 for the wall. Every papilla examined bore some taste buds, whereas 20 per cent of the walls lacked them. The distribution of taste buds is uneven, often localized, and without any common pattern.

Combining our results on individuals between 20 and 69 years, with data obtained by Heiderich for individuals between birth and 20 years, gives a normal mean value of 228 buds to each papilla and 47 buds to each trench wall. The corresponding modes lie between 251 and 300 for papillae and 1 and 50 for trench walls. Probably these several values approximate the truth for these highly variable elements as well as can be expressed at present.

99. *Development of an aglomerular pronephros.* Philip B. Armstrong, Department of Anatomy, Cornell University Medical College, New York City.

The early differentiation of the pronephros in the toadfish (*Opsanus tau*) is essentially the same as in *Fundulus majalis*, which has been described in detail by the author. In both species the cephalic end of the pronephric anlage forms as a primary fold, which is subsequently separated from the coelomic mesoderm by the secondary fold. The archinephric duct anlage continues caudally from the posterior end of the primary fold as a solid cord of cells. A luminal system forms shortly after the separation of the primary fold from the lateral mesoderm. An interesting feature of the later differentiation in the toadfish pronephros is the non-development of a glomerulus. The cephalic end of the pronephros becomes closely apposed to the dorsal aorta and the cellular alignment sometimes suggests a pronephric chamber, but no evagination from the aorta into this mass of cells occurs. This is the mode of formation of the glomerulus in *Fundulus*. In later development the close apposition of the cephalic end of the pronephros to the dorsal aorta is lost.

Marshall and Smith attribute the absence of glomeruli in the mesonephros of *Opsanus tau* to the influence of a marine habitat on its development. Apparently, the pronephros is likewise susceptible to this influence, as it shows a complete suppression of glomerular development.

100. *Development of the gonads following early removal of the hypophysis in Rana sylvatica.* Wayne J. Atwell, Department of Anatomy, University of Buffalo.

Following hypophysectomy at the tail-bud stage in *Rana sylvatica*, the gonads develop in a manner very similar to the normal for a considerable period of time. Even after metamorphosis there is little to distinguish the ovaries or testes of operated from those of unoperated animals. At 40 days after operation, the size of the testes of silvery (hypophysectomized) tadpoles, as determined by Hammar's method equals 6.9 units; compared with 9.1 units for the normal at 48 days. At 71 days the testes of the silvery average 14 units against 13.6 for the normal. At about 170 days, however, the testes of the silvery animal average 39.3 units and those of the normal 91.8 units.

A similar condition obtains with reference to the ovary. At 72 days after operation the ovaries of silvery tadpoles average 131 units compared with 113 units for the normal; while at about 180 days the ovaries of silvery animals average 573 units and those of the normal 1181 units. (The unit here employed for the ovary is one-quarter the size of the testis unit.)

Thus it is apparent that early removal of the hypophysis is not incompatible with a very considerable degree of development of the gonads in this amphibian. It is not until several months have elapsed that significant size or structural differences may be detected in favor of the unoperated animal. Essentially similar conclusions have been reached by Humphrey (unpublished data) for *Amblystoma tigrinum*.

101. *Golgi apparatus of the ameloblasts and odontoblasts of the rat.* H. W. Beams and R. L. King, State University of Iowa. (Introduced by F. A. Stromsten.)

The odontoblasts and ameloblasts of the developing molar teeth of a 2-day-old rat have been prepared according to the method of Nassonov for the demonstration of the Golgi apparatus. In the odontoblasts the Golgi apparatus is a well-formed net and is typical of mammalian glandular cells in that it is polarized between the nucleus and the distal end of the cell. In the ameloblasts, however, no such marked polarity exists; the Golgi apparatus, which here is relatively less abundant and simpler, varies in position, usually being located between the nucleus and the base of the cell or in a perinuclear position. A definite lack of polarity for the Golgi apparatus of both the ameloblast and the odontoblast has been reported by Massenti. It is clear that a definite polarity exists in the odontoblasts; in the ameloblasts, however, the condition has not been finally determined in relation to the formation of enamel.

102. *Feulgen's 'nuclealreaktion' and blood platelets.* H. W. Beams and R. L. King, State University of Iowa. (Introduced by F. A. Stromsten.)

In normal human blood smears treated by the Feulgen 'nuclealreaktion' for the demonstration of nucleoproteids the nuclei of the various types of leucocytes color beautifully. However, the reaction is distinctly negative as regards the so-called chromomeres ('nuclei') of blood platelets. If Feulgen's method can be considered a specific test for chromatin material the evidence seems conclusive that blood platelets represent neither nucleated cells nor the remains of the extruded nuclei of erythrocytes. Furthermore, supravital preparations of blood stained by Janus green B show granular bodies in the platelets comparable in every way to granular mitochondria. The mitochondria are similar to those in the cytoplasm of the megakaryocytes; this might be considered evidence to support the theory of Wright that blood platelets are derived from the megakaryocytes, and not from precipitation products of the blood plasma nor from degenerating erythrocytes.

None of the erythrocytes of normal human or rat's blood stained by Feulgen's method show 'reticulated substance' or bodies comparable to those of Howell-Jolly or Isaacs. This evidence would seem to be in harmony with the view that in normal blood the nuclei of the normoblasts are extruded instead of giving rise to the reticulated substance of the erythrocytes by undergoing intracellular karyolysis.

103. *Total growth and body proportions as influenced by pituitary rudiment implantation and extirpation in urodele embryos.* Raymond F. Blount, University of Minnesota.

Urodele larvae developing with the excess pituitary tissue of transplants made during the larval period show marked growth changes. The results are based upon 203 triple pituitary animals, 223 controls, and 73 hypophysectomized animals. These have been studied by measurements taken at intervals from about 5 days before feeding begins to metamorphosis.

As measured by total length and by volume, the triple pituitary animals are greatly reduced in total growth. This is also true of the hypophysectomized individuals to a lesser degree. These changes begin before feeding and progress with age.

In the study of body proportions, eighteen different measurements have been used. The value for each body part is expressed as a percentage of the trunk length. The rate of growth for each part has also been determined.

The triple pituitary animals show a disproportionate reduction in tail length, head length, head width, tail thickness, and height of caudal muscle. This reduction is extreme for the peripheral parts, such as gills, digits, and height of caudal fin.

In the hypophysectomized animal most of the body parts, although reduced, retain their normal proportions. This is not true of the peripheral parts, such as gills, digits, and tail fin. It is interesting that the latter are markedly increased, while in the triple pituitary they are greatly reduced.

104. *Induced maturity of the reproductive tract of salamanders through the agency of the gonad stimulating principle of the anterior hypophysis.* R. K. Burns, Jr., and Adrian Buyse, School of Medicine and Dentistry, University of Rochester.

Previous experiments, already published, have shown that the gonads of larval salamanders, for several weeks prior to metamorphosis, can be stimulated by extracts of the anterior hypophysis to grow much more rapidly than those of control specimens, with average increases in size of 400 per cent, in the case of male larvae. In females growth of the ovary is relatively slight, but a considerable number of follicles contain oocytes far advanced in growth.

Similar treatment after metamorphosis induces even greater response, in the ovary as well as in the testis. There is considerable quantitative variation in both sexes, but in all cases the response is marked. Complete spermatogenesis is found in all experimental testes, and all ovaries show rapidly maturing ova containing yolk and pigment. In females the growth of the oviduct is striking, with close correlation between the size of the oviduct and the number of maturing ova. In males there is the same correlation between growth of wolffian duct and cloaca, and the progress of spermatogenesis in the testes. It is apparent that the capacity of the ovary to respond, is greatly increased in the post metamorphic period.

Photographs, photomicrographs, and slides will be shown. (See demonstration no. 8.)

105. *The extra-ocular mechanism of Opisthoproctus soleatus.* H. S. Burr, Yale University School of Medicine.

The rare deep-sea fish *Opisthoproctus soleatus*, presents two dorsally directed telescope eyes in close apposition. Examination of microscopic sections through the orbital region reveals the full complement of muscles of the vertebrate eye, together with their cranial nerves from their deep origin. The IV nerve supplies the homologue of the superior oblique. Its action must be to tip the eye forward. The III cranial nerve supplies four muscles: a posterior homologue of the superior rectus which tips the eye backward; an internal rectus which rotates

the eye about its vertical axis; a pair of ventral muscles which assist in the tipping movement of the eye. There is no ciliary ganglion and no ciliary nerve, since there is no iris diaphragm nor true ciliary body. There is a well defined ganglion peripherally placed upon the III nerve from which a non-medullated trunk proceeds to a second ganglion just above the basi-sphenoid. This is connected by a strongly developed nerve which unites with a portion of the glossopharyngeal. Another branch from the III nerve joins the palatine branch of the V. The VI nerve supplies an external rectus which arises in a tunnel in the pre-otic bone, turns sharply about its cephalic margin, and wraps around the postero-lateral waist of the eyeball to be inserted on its lateral aspect.

106. *Branches of the aortic arch in a series of Primates.* Harriet M. Carpenter and C. F. DeGaris, Johns Hopkins University.

The usual human arrangement of aortic branches (anonyma, carotis communis sinistra, subclavia sinistra) was found in: 2 Tarsius, 1 Cebus, 1 Ateles, 1 Pithecius, 3 Pan, 1 Pongo, 1 Gorilla. The gorilla (juv. ♂) had, in addition to the usual human arrangement, a sizable branch from the arch between anonyma and carotis communis sinistra directed ventrally to pericardium and thymus. The arch having a common root for anonyma and carotis communis sinistra was found in: 1 Pithecius, 1 Saimiri, 1 Callithrix. The arch having a common trunk for anonyma and carotis communis sinistra, with the latter given off beyond the subclavia dextra, was found in: 1 Lasiopyga, 1 Pithecius; the same, with carotis communis sinistra given off first, was found in: 6 Lasiopyga, 4 Ateles, 1 Cerocebus, 1 Nycticebus, 1 Hylobates, 1 Pongo, 9 Pithecius rhesus, 6 P. sinicus, 2 P. mordax, 2 P. irus. A bi-anonymous arrangement, with long right and short left common trunks, respectively, for right and left subclavian and carotid arteries, was found in 1 Pan. The a. thyreoidea ima was found only in Pan, in 2 cases as a branch from the root of the carotis communis sinistra, in 1 case as a branch from the arch between anonyma and carotis communis sinistra. The arch with branches completely clumped (common root and trunk) occurred in: 1 Ateles, 1 Perodicticus. (Nomenclature after D. G. Elliot, '13.)

107. *The gonadotrophic hormone of pregnant mares.* H. R. Catchpole and W. R. Lyons, Division of Animal Husbandry and Department of Anatomy, University of California. (Introduced by Herbert M. Evans.)

Following the work of Cole and Hart ('30-'32) on the gonadotropic hormone of pregnant mares' blood, we have investigated quantitatively its distribution in maternal and fetal horse tissues. Chorion, endometrium, fetal and maternal sera and hypophyses at all stages of gestation were assayed on 21-day-old female rats. At first, straight sera and saline extracts of tissues were used, but later a uniform procedure of making powders from acetone-ammonia extractions in accordance with the method of Evans, Meyer, and Simpson was adopted.

Blood serum rises to maximal activity between the 2" and 5½" fetal stages, diminishes thereafter and disappears at about 12½" (i.e., is not detectable at 1000 mg. dosage). Hormone concentration in endometrium is already exceedingly high at 1" (1 mg. endometrium here comparing favorably with 100 mg. serum)

and remains high until 4", then falls and disappears at about 10". Chorionic preparations, while occasionally very potent as early as the 2½" stage, reach maximal activity at 4" to 8" (then being usually more potent than maternal serum) and are inactive after 14". From ½" to 8" fetal stages, maternal hypophyses show rather uniform activity, 100 to 200 mg. glands representing borderline activity. Hypophyses from 8" to 36" stages, and from non-pregnant mares are rarely active at this level.

The evidence appears balanced between hypophyseal and chorio-endometrial origin of this gonadotropic hormone; further study of pre-implantation stages should be crucial.

108. *The lactation hormone of the hypophysis.* H. R. Catchpole and W. R. Lyons, Department of Anatomy, University of California. (Introduced by Herbert M. Evans.)

Before Riddle published his note on 'Prolactin' (1932), we had found that lactation in ovariectomized virgin rabbits could not be produced by growth hormone, by 'prolan,' nor by a combination of these. Potent gonadotropic extracts of pregnant mare serum likewise were ineffective. Extracts of hypophysis, free of growth, but not of gonadotropic hormone, gave good lactation. Using Riddle's method, we later freed the lactation factor of the hypophysis from the gonad-stimulating factor.

We used 5½-month-old rabbits isolated at weaning, showing ripe ovaries, but no corpora, and mature mammary glands, but no milk. Both ovaries and a control mammary spread were removed on the day injections were begun. Study of serial sections of all ovaries has convinced us that Corner ('30) is right in stating that corpus luteum formation is not a prerequisite for lactation. But these ovaries always showed a glandular tissue, composed of those theca-interna cells which normally contribute to the formation of corpora. Extracts of sows' corpora (Fevold, '32), with which we succeeded in 'sensitizing' male rabbits to the lactation factor, may possibly contain a secretion of these cells.

Adult ovariectomized rabbits appeared more reactive to the lactation hormone if previously exposed to 'lutein sensitization,' but extensive duct and alveolar development with lactation followed hypophyseal treatment alone.

109. *Duplicated tectal connections in fishes.* H. H. Charlton, University of Missouri School of Medicine.

It is well known that fasciculi of the optic nerve and the brachia of the corpus geniculatum reach both the rostradorsal and the caudolateral poles of the optic tectum. Monocanthus has also, 1) a brachia tecti cruciatus, which runs from one geniculate through the commissura intergeniculatus to the opposite side, thence to the dorsal level of the nucleus anterior thalami, where it becomes larger and loses its Weigert staining qualities. From the mass two bundles arise and pass to the two tectal poles to terminate similarly to the brachia proper. A nuclear synapse in the non-staining mass seems unlikely. 2) Contrary to the opinion of some workers, large bundles connect the nucleus pretectalis and the tectum passing in a comparable manner. Fibers have been observed, in silver preparations, passing directly from cells of the nucleus into

these tracts. 3) The nucleus preopticus pars recessi gives off a very large myelinated bundle to the lateral tectal pole and seemingly some fibers pass to the dorsal pole as well. 4) One of the described connections of the commissura horizontalis is with the dorsal tectum. Here, in addition, one finds a lateral limb coursing from the commissure to the deep tectal layers of the lateral pole. 5) The ganglion isthmi sends fibers to the dorsal pole of the tectum as well as the previously recorded tracts to the lateral pole.

110. *Thalamic homologies in fishes.* H. H. Charlton, University of Missouri School of Medicine.

Jeener ('30) has attempted to homologize the pars dorsalis thalami with the dorsalis thalami of Amphibia. Evidence supporting this thesis is offered by the finding in the teleost *Monocanthus* of a large, well medullated tract which seems to be homologous to the tractus thalamo-frontalis described by Herrick ('17) in *Necturus*. However, the tract runs here with the median forebrain bundle instead of with the lateral, as in *Necturus*.

111. *A biochemical explanation of sex determination.* F. E. Chidester, West Virginia University.

It has long been known that males have higher combustion rates.

Our own experimental studies have emphasized the role of the unsaturated fatty acids in seizing iodine in the body.

In embryos in which the single somewhat flocculent X chromosome of the egg is augmented by a similar X chromosome carried by the sperm, it is possible that oxidations may be less rapid than in the embryos possessing a single X chromosome in each cell. Carter's studies on iodine-induced parthenogenesis and our knowledge regarding the role of adrenal tumors in producing suprarenal virility in the young female give some support to this hypothesis.

112. *Cytological studies on the new growth of blood capillaries in the living mammal.* E. R. Clark, E. L. Clark, and R. G. Abell, Department of Anatomy, University of Pennsylvania.

Microscopic observations on living growing blood vessels in various types of transparent chambers inserted in rabbits' ears have shown that new vessels extend into the thin double-walled observation space as a growing zone of richly anastomosing capillaries continuous with circulating vessels of the surrounding preformed tissue. Due to the delicacy of the young endothelium, the growing edge of the plexus was often obscured by haemorrhages, making visibility difficult. More recently, using 'round table' and 'moat' chambers with detached splints, more protected regions were obtained in which finest details of the growing capillaries could be seen with the following results:

New blood capillaries of the rabbit grow from preexisting vascular endothelium by a process of sprouting similar to that observed in the tadpole's tail.

The growing capillary tip is closed and may extend as a long fine thread or as a short blunt solid process.

The lumen advances into the tip by gradual extension peripherally or by vacuolization of the solid portion.

Mitoses of endothelial nuclei have been watched and migration of nuclei in the vessel wall.

Fibroblasts which come in contact with the outer wall of new capillaries frequently flatten out and form adventitial (Rouget) cells.

New blood capillaries may penetrate a fibrin network. The fibrin dissolves, leaving a clear space surrounding the vessel as soon as blood enters the sprout.

113. *Innervation of the blood vessels of the central nervous system.* Sam L. Clark, Department of Anatomy, Vanderbilt University School of Medicine.

Using serial sections of the brain stem of a human fetus stained with pyridin-silver method of Ranson and an adult human brain stem and the brain stem of a sheep prepared by the Pal-Weigert method, nerves have been traced from Pial blood vessels along the vessels penetrating the central nervous system. Such nerves have been followed on vessels within the medulla, mesencephalon, and prosencephalon. The arrangement of the nerves on the vessels of the medulla is like that described in the cat and dog (Clark, '29). The description of the nerves on the vessels of the mesencephalon and prosencephalon is similar to that given by Penfield ('32) for vessels teased from the cerebrum. The Pal-Weigert series show that relatively few of the nerve fibers along the vessels of the central nervous system are myelinated as compared with a proportionately greater number on the vessels of the pia mater. Nerve fibers and endings have been observed in the chorioid plexuses of the ventricles in both human and sheep brains. Examples of the regions in the human brain to which nerves have been traced on the blood vessels are: between the olive and pyramid, near the nucleus of the third nerve, near the red nucleus, lateral to the third ventricle, in the thalamus, in the caudate nucleus, adjacent the internal capsule, beneath the insula, etc.

114. *A case of hereditary absence of the distal interphalangeal joint of the index finger.* Arch E. Cole, Department of Anatomy, University of Louisville School of Medicine.

The literature contains reports of many cases of inherited absence of the proximal interphalangeal joint (symphalangism), but only a very few cases of familial recurrence of a defect in the distal interphalangeal joint have been reported. The apparent rarity of the latter condition justifies the recording of the following case.

The anomaly is exhibited in a mother, her son, her daughter (one son is normal), and in five of her first cousins. The defect did not appear in any member of her mother's generation. Two cases, the mother and one cousin, have the defect on the right hand only. The length of the index finger of all cases that could be examined is within normal limits. The distal phalanx is united to the middle one in a straight line.

Skiagraphs of the hands of the son show that the distal phalanges of both index fingers are normally formed. The defect is limited to a fusion of the adjacent ends of the middle and distal phalanges. Trabeculae can be followed from one to the other. In the mother, the distal end of the right index finger is much smaller than normal. The lateral enlargements of the phalanges at the site of the joint are absent.

There are not enough instances of the defect to justify any conclusion concerning its mode of inheritance.

115. *Maceration and resorption of rat fetuses.* E. L. Corey, Physiological Laboratory, University of Virginia Medical School.

The death of rat fetuses was effected by, 1) manual separation of the placenta from the endometrium and, 2) by removal of the fetuses to the maternal peritoneal cavity. Ten to 16 days later, the uteri with their contents, or, in the case of (2), the fetal remains in the maternal body cavity, were removed for histological study. Tissue degeneration was first observed in the fetal liver and gastro-intestinal tract, indicating the fetal origin of the lytic (macerative) enzymes. Later, the spleen, pancreas, and kidney become involved. Cartilage was found to resist autolysis, and considerable amounts of calcium were deposited in this tissue as is common in such material. Skeletal muscle and skin degeneration occurred late in the lytic process. The uterus and amnion act as mechanical barriers protecting the fetus from enzymes of maternal origin, and also serve to protect the mother from toxic substances liberated during fetal retrogression. The presence of dead near-term fetuses (30 to 35 mm.) within either the uterus or maternal peritoneal cavity resulted in the death of the mother in from 1 to 3 days. The role of the maternal leucocytes in the resorptive process is evidently limited to the removal of fetal debris. Other maternal leucocytic reactions to necrosing fetal tissue were found to be similar to those occurring in man, as evidenced by selected clinical material.

116. *The nuclear configuration of the subthalamus and hypothalamus of Macacus rhesus.* Richard L. Crouch, University of Missouri Medical School. (Introduced by H. H. Charlton.)

Brains fixed in trichloroacetic acid and stained with toluidine blue according to the technic of Huber were used in this study. No study was made of fiber stained material, as the purpose of this work was to study the grouping of cells into nuclei as a basis for later study of fiber connections, and for experimental work. Four nuclei in the preoptic region, twenty-one in the hypothalamus and the well-known areas of the subthalamus were identified. The nuclei in *Macacus* conform very closely to those described for lower mammals by Gurdjian ('27), Rioch ('29), Ingram, Hannet, and Ranson ('32), Papez ('32), and others, but in many instances they are not as definitely defined as they are in the lower mammals. This conformity is striking, because it is so much greater in the case of the hypothalamus and subthalamus than it is in the thalamus.

117. *Racial differences in the reaction of developing feathers to artificially administered hormones.* C. H. Danforth, Department of Anatomy, Stanford University.

Since several hormones derived from mammalian sources have proved effective on birds and amphibians, where they elicit responses quite different from those which they induce in mammals, it would appear that these hormones are probably phylogenetically older than some of the reactions which they condition. On

such an assumption the evolution of reactivity to a hormone becomes a matter of interest. This question has been studied in the responses of feather follicles in undisturbed and in grafted skin of the fowl. The results indicate that the reactions are determined by the tissues, and not by the hormones. Whether theelin, thyroxin, or pituitary extract will cause a change in color or morphology of a feather depends on the genetic make-up of the individual tested. For example, such an amount of theelin as a bird of one genotype will utilize to produce a definite effect may appear to be entirely inert when administered to a bird of another genotype. In view of the apparent similarity of wild species of this genus, the marked differences in response of various domestic races to the same hormone have probably all developed rather recently. Consequently, it seems likely that in this species racial idiosyncrasies in relation to hormones have come about by more or less abrupt changes, rather than by a gradual evolution.

118. The arteries at the base of the brain in American white and negro stocks.

C. F. DeGaris, Johns Hopkins University.

In 198 dissections of the basal arteries of the brain (85 whites, 113 negroes) the following categories of variations have been recorded: I) a. basilaris deviating grossly from midline (52 W., 11 N.); aa. vertebrales separate to upper border of pons, there forming short a. basilaris (2 W.); 'island' formations in a. basilaris (2 W.); loop from a. basilaris surrounding right n. abducens and giving off a. auditiva int. and a. cerebel. ant. inf. (1 N.); a. vertebralis of one side continuing as a. cerebel. ant. inf. (and a. auditiva int., 1 case) with or without slender contribution to a. basilaris (3 W., 1 N.); aa. vertebrales grossly unequal in size (46 W., 55 N.); II) a. communicans ant. double (7 W., 2 N.); giving off median ventral ramus (2 W., 1 N.); giving off dorsal ramus (3 W.); replaced by retiform arrangement (1 W.); definitely lacking (1 N.); III) a. communicans post. unilaterally large, continuing as a. cerebralis post. with slight or no contribution from a. basilaris (22 W., 10 N.); same bilaterally (2 W.); replaced by a. chorioidea (1 W., 2 N.); lacking on one side (5 W., 2 N.); lacking on both sides (1 N.); IV) a. cerebralis med. dividing near its origin into two large rami (2 W., 2 N.); V) loop from a. basilaris surrounding right n. oculomotor., and giving rise to a. cerebralis post. and a. cerebel. sup. (1 W.). Often, especially among whites, more than one variation occurred in the same specimen.

119. A comparative study of the lateral reticular nucleus in the upper three cervical spinal cord segments. Leonard C. DeLozier, Anatomical Laboratory, Northwestern University Medical School. (Introduced by William F. Windle.)

The area of gray and white matter at several representative levels in each of the upper three cervical cord segments has been computed for the following animals: frog, chicken, alligator, opossum, rat, rabbit, sheep, dog, cat, monkey, and man. The ratio of gray to white matter in all segments is greater in the rat and rabbit than in other forms. The increase in this ratio at C1 over that at C2 and C3 is greater in the rabbit, dog, and cat than in the other forms and is least marked in the rat. The increase is due to the presence of a very marked

lateral reticular nucleus in the first cervical spinal segment. This nucleus is of least prominence in the animals which lack a pyramidal tract. In the rabbit, whose pyramidal tract appears to end at the upper end of C1 (Swank, '32), the reticular nucleus is extraordinarily large in that small region only.

120. Reactions of immature monkeys to injections of extracts of pregnancy urine.

A. W. Diddle and Edgar Allen, Department of Anatomy, University of Missouri.

Four females, ranging in age from 12 to 18 months and in body weight from 1740 to 2600 gm., and one male, 8½ months of age, weighing 1515 gm., were injected for periods ranging from 20 to 30 days. Previous to injection, 3 females were semiovariectomized and the male semicastrated.

Injections of follutein (Squibb) were made twice daily. A daily dosage of 20 to 30 mouse units was gradually increased to 90 to 100 units in 11 to 19 days, and the maximum continued for 11 to 12 days. The total mouse units of follutein injected ranged from 1443 to 1832.

Findings in the females were as follows: No reddening of the sexual skin, little vaginal epithelial growth, little growth of uterus beyond the infantile condition except in the two heaviest animals in which slight subepithelial hemorrhage occurred, little ciliation of fallopian tubes. Stimulation of growth of ovarian follicles was not marked. In addition to the usual atresia of follicles of all sizes, a considerable number of the larger follicles showed dark, prominent outer epithelial layers.

In the male the remaining testis, normally in the abdomen at this age, descended prematurely into the scrotum. It showed increased size of tubules when compared to the control.

No marked changes were apparent in thymus, hypophysis, thyroids, parathyroids, or suprarenals.

121. The stainability of Nissl material in adrenalectomized rats. J. C. Donaldson and Ira D. Hogg, Department of Anatomy, University of Pittsburgh School of Medicine.

In the Jordan and Ferguson Histology ('16, p. 124), the statement appears that in brains of animals deprived of adrenin the Nissl substance does not stain. This is based on unpublished work by Dr. G. W. Crile. Since adrenin is a product of the chromaffin system and since in the rat the chromaffin cells are confined to the adrenal glands, this form is a favorable one on which to test whether this failure to stain is a general result of loss of adrenin. Albino rats of both sexes were adrenalectomized. Some were examined within a week of operation while in apparent good health and others at a later time when in extremis from the effects of adrenal insufficiency. The staining properties of the Nissl granules varied somewhat in these animals, but no more so than in the controls. In no case was there complete loss of stainability. In this form, evidently, loss of all the chromaffin tissue does not markedly affect the staining capacity of the Nissl material. (See demonstration no. 11.)

122. *Morphology of unmyelinated nerve fibers.* T. C. Douglass, Northwestern University. (Introduced by H. A. Davenport.)

Microscopic study of the morphology of unmyelinated nerve fibers fixed in formalin-acetic and stained by alcoholic silver nitrate (Davenport, '30) showed that these fibers are grouped into bundles which accompany an endoneurial strand that resembles a band fiber. This grouping was much the same in all material, which included vagi from turtle and cat, dorsal roots from cat and rabbit and peripheral mixed nerves from cat. The morphology of unmyelinated fibers was sufficiently characteristic to permit counts (checked by osmic acid stains) of myelinated fibers to an accuracy of ± 5 per cent. The feasibility of counting both myelinated and unmyelinated fibers from silver stains confirms work of Ranson and Helen K. Davenport ('31).

123. *The diameters of dorsal root fibers in some common mammals.* Donald Duncan, Department of Anatomy, University of Texas.

The diameters of all myelin sheaths in sample areas of the thoracic dorsal roots from mature rats, cats, and cows were measured from photographs of osmic acid preparations. The results indicate that there is no natural grouping of fibers into classes of various sizes; i.e., large, medium, and small. Diameters from the cow range from 1.5 to 23 μ ; rat, 1.5 to 16 μ ; and cat, 1.5 to 15 μ . Fibers of all intermediate diameters occur in each species. The distribution of diameters is unimodal in all cases. In the cow 45 per cent of the dorsal root fibers are less than 3.6 μ in diameter; in the cat, 25 per cent; in the rat, 15 per cent; less than 2.6 μ : cow, 23 per cent; cat, 9 per cent; and rat, 7 per cent. Study of both osmic acid preparations and silver preparations for axis cylinders indicates that the high percentage of fine myelinated fibers in the dorsal roots of the cow is due to the acquisition of myelin by fibers that remain unmyelinated in the cat and the rat.

124. *Studies in anatomy of the heart.* Thomas Horace Evans, Long Island College of Medicine.

a. *Corpus spongiosum vel cavernosum metacardiacum:* The deep cervical fasciae extending into the thorax, investing the superior venae cavae, contain around these large veins some spongy tissue, infiltrated by plasma or venous blood, more in man than in comparative studies, and furnishing a suggestion or similarity to the structure of the sinus cavernosus of Willis, and of the corpus spongiosum or cavernosum penis. It may act to support the contractions of the heart and diminish vibrations, and is supplied by branches of the phrenic nerve and ansa cervicalis.

The diaphragm, the capsule of Glisson, the umbilical vein, the suprarenals (in part), the pericardium, the pleura, the peritoneum, and a congeries of tissues mesoblastic in character are related to the phrenic. This is contrasted with the vagus going to the lateral skin (reduced to external auditory area in man) to the alimentary tube as far down as the ileo-caecal valve, possibly to testis and to ovary, and to solar plexus, to larynx, and to lung.

b. Left anterior papillary muscle of the right ventricle: Two implantation types are shown, with some relation to the blood and nerve supply. Action of tricuspid leaflet may exert influence upon the two types of implantation, as in one the muscle is based on the middle of the front of the ventricular wall, and the second shows the muscle is firmly planted on the cardiac septum. (See demonstration no. 12.)

125. *Observations on the genito-urinary tract.* Thomas Horace Evans, Long Island College of Medicine.

a. Ureteral vein from right ureter extending 30 or 40 mm. upward and to the left to the inferior vena cava: Many such examples have been dissected and used in postgraduate courses as well as found by the undergraduates in the Long Island College of Medicine.

b. Four fascial cylinders of human shaft of penis. A fourth cylinder surrounded by fascia related to fascia of Scarpa is seen in section of shaft of human penis made just above ligamentum fundiforme penis (G. A. Piersol), which conveys the deep dorsal vessels and the nerves. This cylinder is deep to the loose areolar tissue envelope given by Cunningham. (See demonstration no. 12.)

126. *Observations on the vascular system.* Thomas Horace Evans, Long Island College of Medicine.

a. Bronchial veins occur in stages of different types or sets of drainage: Three types of drainage of these veins show that the vein (or veins) drain into the azygos, or into the azygos system and also into pericardiac or mediastinal, and, thirdly, into the latter alone. A certain possibility exists of modification genetically, but also of pathological modification.

b. Branch of recurrent laryngeal (left) passing into area of vestigial fold of Marshall and uniting with a branch from the phrenic nerve (left) to supply area of left superior vena cava: This branch exists in many dissections more or less easily recovered by cutting down upon the vestigial fold and exposing the parallel remains of a fibrous cord or patulous vein, and tracing upward and downward. A loop between the branch of the left phrenic nerve here and a branch of left recurrent laryngeal nerve is seen. Comparison with supplies from right phrenic nerve confirm text.

c. Lymph nodule near cystic duct: Note of recovery of small nodules (one to a body) resembling that described as a lymph nodule of Hideo Enomote. Area involved considered as a drainage unit. (See demonstration no. 12.)

127. *Uterine motility of the rabbit, with special reference to corpus luteum inhibition to pituitrin.* Mark A. Foster, University of Wisconsin. (Introduced by M. F. Guyer.)

In vitro studies have been conducted on rabbit uteri treated with the various corpus luteum fractions developed in this laboratory. These preparations obtained from pig corpora represented all stages of purification from the crude to the crystalline corporin, including all residual fractions. Residues from the various stages in the purification process contain small quantities of both the

inhibiting material and corporin. Results show a gradual loss of the pituitrin inhibiting reaction as the extracts are subjected to higher purification, culminating in its absence in the pure crystalline corporin. Uteri treated with the crystalline material show a maximal progestational modification and yet give a maximal pituitrin response.

128. *Regeneration of the ear in Amblystoma punctatum.* Margaret Shea Gilbert, Wellesley College. (Introduced by Helen W. Kaan.)

The auditory anlage was removed from *Amblystoma punctatum* embryos of the closed medullary fold stage, previous to the appearance of the auditory plate. The embryos were preserved at short intervals for 2 weeks following the operation. Thus a close series of embryos showing successive stages of regeneration of the ear was obtained.

It was found that the wound made by the removal of the auditory anlage is soon filled with neural crest cells. A new auditory plate is formed which seems to have arisen from the joining of the ectoderm surrounding the wound with these neural crest cells. Thus, although the normal ear forms from the ectoderm alone, the regenerated ear may develop from neural crest cells and ectoderm.

Closure of the regenerating otic cup occurs by the ventral growth of the dorsal lip, whereas in the normal ear the ventral lip of the cup grows dorsally. In other respects the development of the regenerated and normal ears is similar. The rate of growth and differentiation of the regenerated plate itself is retarded, but later, during its invagination, marked mitotic activity is observed in it. This causes an increase in the rate of differentiation of the regenerated as compared with the normal ear, with the result that at the time of completion of the auditory vesicles, the two ears are similar in size and shape.

129. *On the role of amino acids in the differentiation process.* F. Gudernatsch and O. Hoffman, Washington Square College, New York University.

In previous experiments, we showed that α -amino acids in 1 and 2 acid mixtures fed, as the only N source, to anuran tadpoles, comprise three groups, each best suited for one particular phase of development. In order of increasing molecular complexity, the groups are: glycine, leucine, alanine (Maintenance); arginine, cystine, lysine (Growth); phenylalanine, tyrosine, tryptophane (Differentiation). The D group is extremely unsuitable for maintenance and growth. Poor maintenance (starvation) naturally is a limiting factor for growth and differentiation. These experiments were extended to from 3 to 9 acids, augmenting each M, G, or D group by 1, 2, 3 acids of the other groups, total acid concentration being uniform.

1) Three acid groups—only the M group was sufficiently well maintained to reach belated differentiation; not exceeding basal diet (carbohydrates, fats, vitamins) until late. 2) Four—earliest and most advanced differentiation in mixtures containing D; noticeable in 3M + 1D, more in 1G + 3D. 3) Five and six—group differences lessened; D mixtures in advance, more with G than M. 4) Seven—2D and 3D more advanced than 1D mixtures; among the latter, tyrosine mixture being superior. 5) Eight—extent of differentiation greatest in 3M + 3G + 2D (tryptophane, tyrosine). This also represents greatest differentiation in the entire experiment. 6) Nine—high rate of differentiation, though extent not greater than in eight acids.

Differentiation acids improve the differentiation value of amino acid mixtures.

130. *Defects in the muscular coat of the human vermiform appendix.* Béla Halpert and Dan S. Egbert, Laboratory of Pathology, Yale University School of Medicine.

The circular and longitudinal muscle layers of the vermiform appendix were studied in specimens received in the Laboratory of Surgical Pathology. They were fixed in formalin and hardened in alcohol. Transverse sections were then taken from the proximal two-thirds and a longitudinal section, in the plane of the mesoappendix, from the distal third. Paraffin sections stained with haematoxylin eosin and by van Gieson's method were prepared.

Defects in the muscular coat regularly occur along the mesenteric border where the medium-sized blood vessels penetrate to reach or leave the submucosa. Variations in the thickness of both the circular and the longitudinal layers in different parts of the same appendix are frequently noted. Defects in each of these two layers are occasionally seen unaccompanied by mucosal herniations.

Diverticula were noted in 11 of the 6038 appendices examined during the past 10 years (January 1, 1923, to December 31, 1932). According to Herb, only 25 cases were reported until 1907. Pooling our numbers with those of Konjetzny ('09), MacCarty and McGrawth ('11), Moscheowitz ('19), Stout ('23), Chase ('27), and Mulsow ('32), 55 appendices with diverticula were encountered in 15,066 specimens. Our more recent observations, however, indicate that diverticula of the appendix are more frequent than would seem from the above data.

131. *A method of transplanting limb buds in chick embryos.* V. Hamburger (Rockefeller Foundation Fellow), Hull Zoölogical Laboratory, University of Chicago. (Introduced by B. H. Willier.)

This report deals with a method of transplanting limb buds in the chick embryo, designed to study the relationship of a supernumerary limb to the differentiating nervous system.

Both donor and host were embryos of 35-40 somites (3 days' incubation), when the limb buds are distinct. The donor blastoderm is transferred to a dish containing warm salt solution and a wing or hind limb bud is cut off with an iridectomy knife. The host embryo is prepared by sawing a rectangular window in the shell above the embryo. The window and the shell membrane are removed and by means of a Spemann glass needle a longitudinal slit is made through the amnion and then through the somatopleure just lateral to the somites lying between right fore and hind limb buds. By means of a micropipette, the isolated limb bud is dropped upon the wound and worked into the slit with a glass needle or hair loop. It is held in close contact to the somites by the elastic somatopleure. The window is sealed in and the egg returned to the incubator. In 38 out of 46 cases which survived 3 days or more after the operation the limb bud healed in and grew. In 13 of these the limb was normal in form and occasionally of normal size; in the remainder abnormal appendages developed. (See demonstration no. 15.)

132. *Studies on the lymphatic vessels and on the movement of lymph in the ear of the rabbit.* C. G. Henry, Department of Anatomy, University of Pennsylvania. (Introduced by E. R. Clark.)

Studies on the lymphatics and on the movement of lymph in the rabbit's ear were carried out by injections of the lymphatics and blood vessels of the intact ear, by the use of injections in conjunction with transparent chambers introduced into the ear, and by the collection of lymph in cannulae inserted into lymphatics of the intact ear. As a result it was found that:

The rabbit's ear possesses a rich lymphatic supply of both superficial and deep vessels, and many collecting vessels, following the course of the main blood vessels.

The lymphatics of the inner side of the ear pass through the cartilage to join those of the outer side.

The total surface area of lymphatics approximately equals that of the blood vessels.

The amount of lymph flow in the normal ear was calculated to be from 0 to 0.0125 cu.mm. per square millimeter of lymphatic surface per hour during spontaneous flow, with a maximum rate of 0.084 cu.mm. during massage.

The 'preformed tissue' chamber offered some obstruction to the flow of lymph at the periphery of its thinner portion.

Granules injected into lymphatics confirm the presence of temporary post-operative openings in lymphatics within the 'preformed tissue' chambers, which may persist as long as 13 days.

133. *The spinal opossum and its reflexes.* J. C. Hinsey and C. C. Cutting, Department of Anatomy, Stanford University.

Five adult opossums were transected in the lower thoracic segments and were kept 2 to 3 months. They ate and drank normally, defecated and urinated spontaneously. Reflex responses in the hind limbs were observed daily. Within 2 hours after recovery from ether, it was possible to demonstrate ipsilateral flexion with extensor rebound from pinching the toes, ipsilateral extension from the great toe, and the Stütz positive reflex from gentle pressure to the plantar surface of the foot. Crossed reflexes did not appear until from 2 to 5 days and crossed flexion and crossed extension were both seen, with strong stimulation predisposing to crossed flexion. Rhythmic stepping appeared in from 4 to 16 days, and the scratch reflex with well-developed local sign in about 18 days. Standing with support of the body weight for about 3 minutes appeared in the second month following transection. Stimulation of the extensor surfaces of the limb and the whole of the great toe generally gave ipsilateral extension, while the flexor surfaces, exclusive of the great toe, elicited flexion. These reflexogenous zones were present after 24 hours.

Kymographic tracings produced by contractions of the isolated gastrocnemius and anterior tibial muscles in the unanesthetized animal showed the complete Stütz positive reflex with cocontraction of the extensors and flexors. Crossed responses in these preparations were difficult to obtain.

134. *Studies on neurological mechanisms in ovulation in the rabbit.* J. C. Hinsey and J. E. Markee, Department of Anatomy, Stanford University.

Attention has been focused upon the effect of removal of various portions of the C.N.S. on the ovulation produced by pregnancy urine. It was possible to remove all the efferent nervous pathways to the ovary without arresting ovulation (Hinsey and Markee, '32). Microscopic examination of these ovaries revealed no abnormality in the ruptured follicles.

Mid-brain rabbits were prepared in such a manner that all of the C.N.S. was removed rostral to a section extending from the rostral border of the superior colliculus to the exit of the oculomotor nerve. The hypophysis was left in situ and injection proved the presence of a blood supply. In one series (five large animals), pregnancy urine was injected after short intervals (10 to 95 minutes) following the removal. Ovulation (average, 8 rupture points) occurred in every instance. In a second series (five large animals) with longer intervals (5 to 6½ hours) following removal, ovulation (average, 7½ rupture points) was present in every case. In a third series (six small animals, 2.0 to 2.2 kg.) follicles ruptured (average, 3½ rupture points) in every animal when the urine was injected from 5 to 10 minutes after removal. These observations demonstrate conclusively that ovulation produced by pregnancy urine injection occurs in 100 per cent of the cases in the complete absence of the hypothalamus when the hypophysis is left in situ.

135. *The paired fins of fishes.* A. Brazier Howell, Department of Anatomy, Johns Hopkins University.

In fishes some groups have two pairs of fins that are clearly pectoral and pelvic, located at opposite extremities of the branchioclacal line (elasmobranchs, dipnoans, ganoids, some teleosts); there may be only a pectoral pair (ostracoderms and antiarchs); only a pelvic pair (arthrodires); or two or even three pairs of fins neither of which seems homologous to pelvics (some teleosts). In the first group there is typically a considerable number of intermembral neuromeres between the fin plexuses and many nerves reach the pelvic fin. In a second group of teleosts, of which the cod is typical, the more posterior fins are innervated by very few nerves, which are in immediate sequence with the pectoral plexus, and it is believed that these fins are not pelvics that have migrated, but are derived from the posterior elements of the pectoral series and have shifted ventrally. Instances even occur (*Polynemus*) in which a third pair of appendages has been derived from the pectoral complex. There is a third group of teleosts (some *Ostariophysi*, *Heteromi*, *Catosteomi*, *Percesoces*), however, in which conditions are intermediate, with abdominal paired fins supplied by many nerves, and these might very well have been derived from a pelvic situation.

136. *Gonadal antagonism in *Amblystoma tigrinum* following extirpation of the pars buccalis of the hypophysis in embryonic stages.* R. R. Humphrey, Department of Anatomy, University of Buffalo.

Extirpation of the pars buccalis of the hypophysis was accomplished at about stage 33 in embryos in which the right gonadic preprimordium had been previously replaced by that of another embryo. Ungrafted hypophysectomized

and normal animals, and grafted animals not hypophysectomized, served as controls. When the gonads in the grafted animals were unlike in sex, the testis in the hypophysectomized animal inhibited the development of the ovary and caused its reduction to the atrophic or freemartin condition much as in animals having the hypophysis intact. The formation of the ovarian sacs (central cavity) was inhibited, and the ovarian cortex was greatly reduced in volume and in older animals contained few or no oocytes. In ungrafted hypophysectomized females the structure of the ovaries was essentially normal, hypophysectomy alone merely preventing development of the oocytes beyond a certain stage. The inhibitory action of the testis upon the ovary must therefore be direct, and not through depression or inhibition of hypophyseal activity.

Absence of the hypophysis in heterosexually grafted animals did not permit the ovary in any instance to escape the modification described above. No ovary under testicular influence was ever found to have regenerated to the condition seen in hypophysectomized female controls, and no testis showed more pronounced development of its cortex than has been observed in males not hypophysectomized.

137. *An experimental study in the determination of the morphology of hydrocephalus, produced in the albino rat when irradiated in utero.* T. T. Job and L. T. Palumbo, Department of Anatomy, Loyola University School of Medicine.

Previous work in this department has established the fact that a definite dose of x-ray when administered to a pregnant rat on the ninth or tenth day of gestation results in hydrocephalic progeny. In studying the morphology of this condition, it was found that the hydrocephalus was of an internal type, with obstruction, which appears to be due to a hyperplasia of the lining ependyma, in the aqueduct of Sylvius. Thinning of the various areas of the cerebral cortex corroborates findings of Weed and Managas. The third ventricle was moderately dilated, while the fourth ventricle, lateral recesses, and foramina of Luschka were apparently normal.

The ventricles of living hydrocephalic animals were injected according to the method of Weed, which further demonstrated the block in the aqueduct of Sylvius, and also revealed, in a few instances, an abnormal opening through the inferior-medial wall of the ventricles which communicated with the basilar cisternae. This communication existed only in older specimens—a fact substantiated by histological study. This possible reestablishment of the flow of cerebrospinal fluid from the lateral ventricles may account for the improvement of the condition in the older animals. (See demonstration no. 27.)

138. *Persistence of a left ischiadic artery.* Thesle T. Job, Department of Anatomy, Loyola University School of Medicine.

Recorded instances are rare of the persistence of ischiadic arteries. Senior has collected records of 13 cases (6 bilateral, 7 unilateral). Adachi and Taguchi each described an added case. An interpretation of such anomalies has been given by Senior ('25). The case here recorded is thought to be the only instance reported in the Americas. It is that of an adult male with persistence of the left ischiadic artery, which is a direct continuation of the hypogastric. It passes

through the greater sciatic foramen, incorporated in the sheath of the sciatic nerve; it supplies the hamstring muscles, becomes the popliteal, and from the superior geniculars distally is typical.

The femoral artery in the proximal one-third is fairly typical in branches and relations and only slightly reduced in caliber. It terminates in the saphenous and musculo-articular branches, without a branch traversing the hiatus. The profunda is typical, except that its two terminal branches, after piercing the adductor magnus, form direct communications with the superior geniculars of the popliteal. The obturator, arising in common with the superior gluteal, is larger than usual, since it is the main supply to the adductor brevis and magnus muscles.

The gross arrangement of these arteries suggests that the obturator developed in the adductor zone in such a manner as to prevent the rete femorale anastomosing with the ischiadica, except through the distal branches of the profunda. (See demonstration no. 20.)

139. *The effects of prenatal injury on certain aspects of development in the rat.*

O. Larsell, Departments of Anatomy, University of California and University of Oregon Medical School.

Fore limbs of rat fetuses were amputated in varying degrees at 16 to 19 days' gestation, to determine the effects on related body parts. Study of the scapulae of operated animals which had come to term shows a reduced development on the side of the fore limb amputation, as compared with that of the opposite side, which served as control. The reduction in size appears proportionate to the amount of injury inflicted and to the stage of development at which amputation was performed. The greater the amount of injury or the earlier the amputation, the smaller was the scapula of the affected side at term. Save in one case, which was omitted from the series, blood vessels supplying the scapula could not have been injured. The result is interpreted as due to reduced metabolic activity in the developing bone because of lessened muscular pull on the bone consequent on destruction of muscle insertions and portions of muscles connecting the fore limb and the scapula.

140. *The superior olivary mass in the frog.* O. Larsell, University of Oregon Medical School.

Ventro-laterally in the medulla oblongata of the frog there is a mass of cells which extends from the rostral level of the IX roots as far forward as the level of the trigeminal roots. In position and connections the mass corresponds, in general, to the superior olive and trapezoid nuclei of mammals. Golgi and Cajal series show that fibers from the dorsal VIII nuclei terminate among the cells, and that these cells, in part, give off axones to the motor VI nucleus and to the median longitudinal bundle. Fibers from the VIII nuclei pass through the cell group to the opposite side, appearing to represent an incipient trapezoid body.

The cells are small, with short dendrites which ramify largely within the nuclear mass itself. They appear derived from the general grisea of the bulb, perhaps drawn to their ventro-lateral position by neurobiotaxic attraction.

141. *A supraclavicularis proprius muscle.* Homer B. Latimer, Department of Anatomy, University of Kansas.

This muscle was found in the well-nourished and well-muscled body of a white male, 51 years old. He had been a laborer at a county poor farm and died of coronary thrombosis.

The muscle, which was on the left side, had a total length of 105 mm. and a maximum diameter of 6 mm. The medial end was attached to the posterosuperior surface of the clavicle 35 mm. from the sternal end and slightly (5 mm.) behind the lateral border of the clavicular head of the sternocleidomastoid muscle. The lateral end was attached to the posterosuperior surface of the clavicle 47 mm. from its lateral end and on the most posterior part of the flexure of the lateral third of the clavicle. The lateral end of the muscle was overlapped for about 35 mm. by the trapezius muscle. The muscle was connected throughout its entire length, with the superficial layer of the cervical fascia.

A space of about 4 mm. intervened between the belly of the muscle and the bone, and through this passed the external jugular vein and the supraclavicular nerves. The vein passed down anterior to the muscle and then between the muscle and the clavicle and on in a normal manner. The nerves passed inferiorly, deep to the muscle, and then came out between it and the clavicle and then followed their normal course. (See demonstration no. 23.)

142. *The stomach contents of a cadaver.* Homer B. Latimer, Department of Anatomy, University of Kansas.

The subject was a well-nourished 16-year-old girl of low-grade mentality, from one of our State Institutions.

The tangled mass of string, coarse thread, and safety-pins was removed, dried in the air, and weighed. There were 140 entire, closed safety-pins, and the head of another. These were all of the same size, being 5 cm. long. In addition, there were 4 grommets, 18 mm. in diameter; a small rock, weighing 0.459 gm.; 1 button 1 cm. in diameter; 2 beads, one an elongated bead, 11 mm. in length, and the other spherical with a diameter of 5 mm., and 4 teeth from a comb. The largest of these was 4×24 mm. and the other three were a little smaller. The entire mass weighed 253.2 gm.

The stomach was distended, but otherwise of normal shape and position. The mucosa did not seem to be injured, although it was much smoother than usual, due to the distention of the organ.

When the mass was taken from the stomach the metal objects were not corroded, which raises the question whether they had resisted the action of the gastric juice better than other objects recovered from stomachs or had they been swallowed but a short time before her death. The records show that she died "unexpectedly of enterocolitis of 5 days' duration." (See demonstration no. 22.)

143. *Genital responses of hypophysectomized female rats and of cockerels to pregnancy-blood serum injections.* S. L. Leonard, R. Kurzrok, and P. E. Smith, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Untreated pregnancy-blood serum has been injected in immature and mature hypophysectomized female rats. Injections were begun immediately after the removal of pituitary or after the lapse of some time. Untreated hypophysectomized litter mates served as controls. From $\frac{1}{2}$ to 1 cc. of serum was injected daily for 9 to 26 days.

These injections caused an enlargement of the ovaries. The structure of these enlarged ovaries is similar to that given by pregnancy urine, there being no follicular growth, but a hypertrophy of the cells of the atretic follicles and of existing cords of interstitial tissue. Although smaller, these cells appear similar to lutein cells.

Injections of pregnancy-blood serum for 10 days into barred-rock cockerels 3 to 4 weeks old elicited but a slight if any response either in the weight of the testes or in the development of the germ cells. The comb growth was within the limits of the variability shown by the controls. The result of the injection of pregnancy-blood serum is thus similar to that of pregnancy-urine which, as shown by Riddle and Polhemus in pigeons, by Schockaert in ducks, and by us in cockerels, does not distinctly stimulate the testes.

144. *Cells and fibers in lumbar nerves.* Victoire Lespinasse, Northwestern University. (Introduced by H. A. Davenport.)

The lowest ratio of nerve fibers in the dorsal root to cells in the ganglion yet observed was found in L2 of dog: 33 fibers to each 100 cells. The proportion of myelinated fibers in the dorsal root of L6 of rabbit was 1 myelinated to 1.6 unmyelinated. In L2 of dog, this proportion was 1 to 0.5; in L6 of dog, 1 to 0.8; and in L7 of cat, 1 to 0.5. In the ventral root of L2 in dog, about 15 per cent of the total number of fibers were unmyelinated. In L6 of dog, L7 of cat, and L6 of rabbit the percentage of unmyelinated was less than 5.

145. *The production in normal living cells of widely split chromosomes similar to those characteristic of malignant tumor cells.* Margaret R. Lewis, Carnegie Embryological Laboratory.

One of the characteristics of malignant cells, demonstrated in tissue cultures by M. R. Lewis, is that during mitosis the chromosomes become more widely split than do those of normal cells. In an effort to arrive at a better understanding of cancerous tissue, attempts were made to bring about a similar condition in living chick embryo cells growing in Locke-Lewis solution.

This was accomplished with least injury to the growing cells by diluting the medium of the cultures with distilled water during mitosis.

The split occurred along the length of each chromosome so that they appeared as pairs of parallel bodies as they do in the cells of the spontaneous mouse cancers. The cells with the experimentally produced split chromosomes completed division and continued to grow.

Dilution of the medium demonstrated the marked difference in size that exists among the chromosomes of a chick embryo cell. Some were long, others short, and some were extremely small granules. When the medium was properly diluted, each chromosome, regardless of size, became widely split so that they resembled the chromosomes of malignant cells undergoing mitosis in tissue cultures.

146. *Studies on the cells of the nervous system, correlated with behavior in acute and chronic morphinism.* E. M. MacEwen and A. R. Buchanan, Department of Anatomy, University of Iowa.

Posterior root ganglia and anterior horn cells. In 43 dogs comparative studies were made on the Nissl substance, neurofibrils, mitochondria, and the Golgi apparatus. The acute morphine dogs were killed 4, 12, and 24 hours after a subcutaneous injection of 50 mg. of morphine sulphate per kg. The tolerant animals had been on a similar dose of morphine from 9 months to 5 years. They were killed 4, 24, and 60 hours after the last dose.

In each case the tissues were compared with those from normal control dogs. No significant alteration was found in either the cells of the posterior root ganglia or in the anterior horn cells of the spinal cord.

Five rats, weighing 160 to 180 gm., received 10 mg. of morphine sulphate subcutaneously, 4 were given an equivalent dose of morphine hydrochloride. Contrary to the report of Ma (Chinese Jour. Phys., vol. 5, p. 251), these rats were not stimulated by the dose. They were asleep in 3 to 5 minutes, rapidly passing into a deep narcosis lasting 3 to 6 hours. The Golgi apparatus from tissues taken at 4 and 9 hours was compared with that in normal rats. Again we failed to find any significant change in the amount or arrangement of this substance. No changes were noted in the shape, position, or size of the nucleus. (See demonstration no. 7.)

147. *Occurrence of typical castration changes in the hypophysis of spayed rats given hypophyseal implants.* Morvyth McQueen-Williams, Department of Anatomy, University of California. (Introduced by Herbert M. Evans.)

Eighteen rat pituitaries stained with orange G-aniline blue were studied in an effort to determine whether in the gonadectomized animal the hypophysis itself might not be affected by treatment with hypophyseal preparations, although the accessory reproductive organs do not disclose any change from the typical castration state. Six spayed animals, each implanted intramuscularly with twenty-two rat hypophyses over a period of 10 days, exhibited on the eleventh day changes in the anterior pituitary similar to that of their untreated ovariectomized litter mates and differing from that of their normal sisters in the increased number and size of the basophiles and the frequent occurrence in these cells of a large central macula, characteristic castration changes.

The findings presented in this abstract seem to confirm in the main almost simultaneous observations on mouse pituitaries stained with hematoxylin-eosin made by Haterius and Charipper.

148. *A note on the effect of thyroid feeding on the numbers of follicles in the ovaries of the albino rat.* Donald Mainland and Basil Kenneth Coady, Department of Anatomy, Dalhousie University.

A preliminary report. Two animals from a litter of four young female albino rats were fed daily for 46 days with dried thyroid extract, for the first 33 days with 0.5 mg. per gram of body weight, for the remaining 13 days with 1.0 mg. per gram of body weight. Each rat, commencing with weight about 70 gm., almost doubled its weight during this period. The kidney weights at death in the two control rats were, for each animal, 1.3 gm.; in the thyroid-fed animals, 2.3 and 2.4 gm., respectively—a proof that thyroid-feeding had affected metabolism. The ovaries were preserved as complete series of paraffin sections. It was found that small follicles (i.e., those without liquor folliculi) were adequately enumerated by counting them in every fourth section and multiplying by 4. For these eight ovaries the average number of this class of follicle was about 3200 per ovary, with variations from 2500 to 3600. (No distinction was made at this stage between atretic and non-atretic follicles.) Other workers have shown that extreme hyperthyroidism causes follicular atresia. The present results indicated that moderate hyperthyroidism did not cause any appreciable change in the number of small follicles (nor, so far as observed, in the numbers of larger follicles), either by altered rate of formation or extreme degeneration and disappearance.

149. *Menstruation in ocular endometrial transplants.* J. E. Markee, Department of Anatomy, Stanford University.

Menstruation was observed microscopically in endometrial transplants in the eyes of six monkeys (*Macacus rhesus*). Vasoconstriction begins 6 to 12 hours before the onset of menstruation and persists throughout the first day. Menstruation did not begin simultaneously in all transplants and sometimes appeared 3 hours before blood entered the vagina. Subepithelial hematmata form and papillae 0.3 to 0.5 mm. in diameter and 0.1 to 0.3 mm. in height appear on their surfaces. A papilla ruptures and hemorrhage continues for 25 to 70 minutes. More hematmata form and rupture and most of the epithelium is desquamated during the first 30 hours. In scanty menstruation only subepithelial hemorrhages occur. In profuse menstruation deeper and large hematmata form and their papillae are lower and usually multiple.

The free blood is lighter than that in the hematmata, but darker than the circulating blood. No blood clots were observed during the first day and only rarely after that around desquamated pieces of endometrium.

Only a small part of a transplant bleeds at one time. No tissue is desquamated during the first few hours and only small bits of epithelium and superficial stroma during the first day. In profuse menstruation some deeper stroma becomes detached after this time.

When reepithelialization was observed, it occurred as an outgrowth of epithelium from the free extremities of the glands.

150. *Studies on the induction of ovulation by prolan in hypophysectomized midbrain rabbits.* J. E. Markee and J. C. Hinsey, Department of Anatomy, Stanford University.

After complete hypophysectomy in midbrain rabbits and short intervals (5 to 45 minutes) between hypophysectomy and pregnancy urine injection, ovulation (average $4\frac{1}{2}$ rupture points in 8 animals) occurred in all cases. After an interval of 3 to $3\frac{1}{2}$ hours (4 animals), ovulation was present in 2 (total of 7 rupture points) and was absent in 2. In 6 animals with longer intervals ($3\frac{1}{2}$ to $9\frac{1}{4}$ hours), ovulation occurred in only 1 (total 2 rupture points). In 6 small midbrain animals (2 to 2.2 kg.) in which the hypophysis was extirpated and urine injected 5 to 39 minutes later, no ovulation ensued.

Ovulation occurs regularly in both large and small midbrain rabbits when the hypophysis is left in situ and when either a short or long interval elapses between removal of the hypothalamus and injection (see abstract no. 134). In hypophysectomized large midbrain rabbits, ovulation occurs regularly when less than 3 hours elapses between hypophysectomy and the injection. When the interval is longer than 3 hours, the incidence of ovulation decreases markedly. In small hypophysectomized midbrain animals, ovulation was absent even after short intervals. It appears that prolan alone is not sufficient to produce ovulation (rabbit) and that the hypophysis elaborates some substance which remains in the body tissues of the larger animals for some time after hypophysectomy and then seems to disappear.

151. *Fiber connections of the cortex of *Pithecus rhesus*.* Fred A. Mettler, Cornell University Medical College, Ithaca. (Introduced by J. W. Papez.)

A Marchi study of the corticofugal connections of the occipital, parietal, temporal and frontal areas of the brain of the Indian macaque following experimentally placed surgical lesions reveals with regard to the striate cortex that: 1) Although there are long association fibers, that these fibers are not arranged into bundles such as the inferior longitudinal fasciculus of Flechsig described for the human brain. 2) The dorsal third of the macular projection area (occipital operculum) is connected with the posterior limb of the angular gyrus and the remaining macular projection area as described by Poliak. 3) The ventral third of the macular cortex associates with the dorsal half of the mid-temporal gyrus and with the remaining opercular area. 4) The middle third of the macular cortex possesses chiefly intraopercular connections and few extrastriate connections. 5) The macular projection cortex is in moderate corticofugal connection with the lateral geniculate body, apparently without regard to the localization segments of this cellular structure. 6) The pulvinar shows chromatolysis following injury of the macular area, but that no significant corticofugal connection can be demonstrated from the macular projection area to this structure. 7) There are no occipitopontine fibers from the macular area. 8) There are no occipitomesencephalic fibers from the macular area. 9) There are occipitomesencephalic fibers from the extramacular cortex (lips of the calcarine fissure).

152. *Capillary innervation from filamentous cells.* N. A. Michels, Daniel Baugh
Institute of Anatomy, Jefferson Medical College.

At the 1932 meeting I demonstrated hitherto undescribed cells found in whole mount preparations of the omentum of rabbits, which I tentatively termed filamentous vasoformative fibrocytes, in view of their association with capillary tuft formation. The facts that the bi- or multiaxial cytoplasmic processes of these cells were extremely narrow (1μ) and long (200 to 950μ), showed syncytial arrangement, and were frequently lost in the capillary wall, induced several anatomists who studied the preparations to suggest the interpretation that the cells might possibly be neurons.

Although five neurological staining methods failed to bring out the nature of the filamentous cells, preparations stained with Maximow's technique present evidence that the cells are related to capillary innervation for an unquestionable origin of an extensive filamentous plexus from a non-medullated nerve fiber ($12,000\mu$) has been observed. Whether the nuclei in the delicate attenuated fibers represent terminal sympathetic ganglia or nuclei of neurolemma cells which accompany non-medullated fibers remains to be determined. Aside from enlargements or flattened expansions of nerve fibers at points of contact with capillary endothelium, frequent abutment of nuclei on the capillary wall, no definite end apparatus on capillaries has been encountered.

Since in most instances the fibers cross the naked endothelial wall and only seldom come in contact with pericytes, the existence of Rouget cells as contractile elements is denied, not only on a histological, but also on a functional basis.

153. *Human variations.* a) *An anomalous subscapular artery.* b) *Triplicate parotid duct.* Shirley P. Miller, Department of Anatomy, University of Minnesota.

a. The right subscapular artery arose from the internal mammary artery 4 cm. below its origin from the subclavian artery. The course of the artery was across the brachial plexus to its normal distribution.

b. Three parotid ducts were found arising from a single gland, and coursed across the face of the buccinator muscle. Two of these fused as they pierced the buccinator muscle. The third entered the muscle and the two ducts opened into the mouth by separate openings.

154. *Implantation, fetal membranes, and placentation in the squirrels (Sciuridae).* H. W. Mossman, Department of Anatomy, University of Wisconsin.

The following material will be demonstrated. Implantation and placentation: fox squirrel (*Sciurus niger*), gray squirrel (*S. carolinensis*), red squirrel (*Tamiasciurus hudsonicus*), chipmunk (*Tamias striatus*), spermophile (*Citellus tridecemlineatus*). Placentation alone: flying squirrel (*Glaucomys volans*), western chipmunk (*Eutamias quadrivittatus*), flicker-tail gopher (*Citellus richardsoni*), woodchuck (*Marmota monax*). The literature on placentation in this family is both meager and inaccessible, consisting of a complete description of implantation and placentation in the European squirrel (*Sciurus vulgaris*), in Dutch, and of the European spermophile (*Spermophilus citellus*) in Czech; also a description

of implantation of the latter in German. In English we have only Doctor Lee's papers on implantation in *Citellus* (*13-lineatus*, *richardsoni* and *franklini*) *Amospermophilus*, *Tamias*, and *Cynomys*, and a brief account by Rau of the placenta of the South African ground squirrel (*Xerus capensis*). From this literature and our material it seems that the sciurid type of implantation and placentation is well represented by *Citellus tridecemlineatus* (see abstract no. 156). In the ground squirrels implantation is by a narrow trophoblastic cone which invades the submucosa, while in the tree squirrels the trophoblast simply replaces the uterine epithelium, but does this over a wide area. The yolk sac, allantoic placenta, and decidua are quite similar in all. The trophoblastic tubules seem to be larger in the ground squirrels and the decidua to be best developed in the tree squirrels. (See also abstract no. 66 and demonstration no. 27.)

155. *Changes in the testes and accessory glands of the gray and fox squirrel (Sciurus carolinensis and niger) during their seasonal reproductive cycle.* H. W. Mossman, A. M. Katz, and L. H. Rubnitz, Department of Anatomy, University of Wisconsin.

The rutting season of these squirrels in this locality is apparently from late December to early July. Since the older females probably raise two litters a year, there is much variability in the breeding activity of the younger animals. The state of activity of an individual male must be judged by examining sections of his testes. The active squirrel testis gives unusually beautiful pictures of spermatogenesis. With testis degeneration the accessory glands undergo atrophy quite equivalent to castration atrophy. The glands of old males, however, never go back to the small size of the prepubertal glands. The atrophic changes seem to be most marked in Cowper's glands and least in the seminal vesicles. Roughly, the extremes of histological appearance in the three chief accessory glands are as follows: Cowper's: Active, fine trabeculae. High epithelium, basal nuclei, 3 or 4 huge secretion granules in each cell arranged serially like buckshot in a shotgun shell. Inactive, very broad trabeculae. Cuboidal epithelium with almost no cytoplasm. Prostate: Active, exceedingly fine trabeculae. Narrow interlobular walls with smooth muscle. Epithelium high with dozens of coarse secretion granules in each cell. Inactive, coarse trabeculae and broad interlobular partitions. Epithelium cuboidal with little cytoplasm. Lumina of secreting tubules practically closed. Seminal vesicle: Active, dilated tubes. Very high columnar epithelium. Inactive, narrow tubes. Low columnar epithelium. (See demonstration no. 28.)

156. *The development of the placenta and fetal membranes of the thirteen-striped spermophile (Citellus tridecemlineatus, Mitchill).* H. W. Mossman and Louis A. Weisfeldt, Department of Anatomy, University of Wisconsin.

Forty-four stages of this ground squirrel from before implantation to full term have been studied. Thirty of the early stages are from the collection of the late Dr. Thomas G. Lee, of the University of Minnesota. As described by Lee, implantation is on the anti-mesometral side. Later, this connection disappears and a yolk-sac placenta is established on the sides of the fixation chamber, only to disappear in turn when the allantoic placenta forms on the mesometral

side. Allantoic vascularization of the placenta begins at the 7-mm. stage. Inversion of germ layers proceeds much as in the rabbit, but technically is never complete, since Reichert's membrane with a few ectoderm and endoderm cells (bilaminar omphalopleure) never disappears. There is a time when its cellular layers are almost absent, but later the endoderm regenerates, even to the extent of forming inwardly projecting, avascular, villus-like outgrowths. The splanchnopleuric portion of the yolk sac is relatively avascular after the middle third of gestation. The placenta is discoidal, labyrinthine, and of the hemochorialis type. The trophoblastic tubules are exceptionally large, averaging about $50\ \mu$ in diameter to about $10\ \mu$ for the fetal capillaries which form a network around them. Decidual tissue is scanty at the base of the placenta, but extends as an externally visible band around the entire gestation sac. (See also abstracts nos. 66 and 154 and demonstration no. 27.)

157. *The effect of large amounts of gonad-hormones upon the anterior hypophyses of normal and gonadectomized rats.* Warren O. Nelson, Hull Zoölogical Laboratory, University of Chicago. (Introduced by Carl R. Moore.)

The anterior hypophyses of gonadectomized and normal male and female rats injected daily for 20 days with 50 R. U. oestrin, an amount far in excess of that necessary for the suppression of castration changes, showed a definite decrease in the number of basophiles and an increase in the chromophobe population. This is the reverse of the condition in untreated castrates. The same picture was obtained in spayed females injected daily with 7.5 B. U. of testis-hormone. The dosages employed to date have not produced this condition in the castrated male.

The above evidence and that presented in the preceding paper indicate that the gonad-hormones exercise a suppressing influence on the basophilic cells of the anterior pituitary. Furthermore, it supports the concept which holds that the basophile derivation from the chromophobes is a reversible one. It is significant that hypophyses from animals treated with oestrin are less potent in gonad-stimulating hormone than are glands from untreated animals, and that gonad-hormone injections have been shown to damage the gonads presumably through a suppressing effect on the hypophysis. This suppressing influence would appear to be due to an inhibition of basophile differentiation and activity. (See also abstract no. 67.)

158. *Development of spontaneous and reflex behavior correlated with the appearance of reflex arcs in the spinal cord of chick embryos.* Douglass W. Orr, Anatomical Laboratory, Northwestern University Medical School. (Introduced by William F. Windle.)

Macroscopic spontaneous motility (simple ventral trunk flexion) begins before 120 hours, simple trunk rotation a little later, and compound lateral flexion ('swimming') at 132 hours. The last begins at the neck and progresses caudad to the leg region, or vice versa. Occasionally, movements begin simultaneously at both regions. Some 132-hour embryos give both local and compound. Separate appendage motions appear spontaneously about the seventh day. Frequency and magnitude of all movements are independent of the intactness of the amnion.

Faradization of trunk and limbs elicits movements conforming to lines of contraction of the stimulated muscle mass in 146-hour embryos. These movements differ from the spontaneous and reflex ones.

Reflexes in response to 'flipping' with a fiber needle appear in the wing at 7 to 7½ and in the leg at 7½ to 8 days. A reflex is a quick lateral extension of the limb followed by a quick return. It may or may not set off a generalized response of trunk and limbs. Usually, any gross disturbances of 7½- to 8-day embryos produces generalized trunk and limb movements. These generalized stimulated movements are identical with the concomitant spontaneous 'swimming movements.'

Study of several embryos stained by pyridine silver shows a well-developed motor apparatus, but a poorly developed sensory one, at 6 days or earlier. At 7 to 7½ days, however, when reflexes appear in the wing, sensory development is marked and sensory collaterals have grown in to complete primitive reflex arcs.

159. *The existence of a 'postmenopause' sexual rhythm in women, as indicated by the study of vaginal smears.* George N. Papanicolaou, Department of Anatomy, Cornell University Medical College, New York City.

The prevailing view, that 'menopause' marks the end of the sexual rhythm in women, is proved to be incorrect in the light of investigations based upon the study of vaginal smears. In all cases examined (up to 10 years after menopause) the sexual periodicity is still present. The sequence of phases follows the same order as in young normal women.

The 'menstrual or destructive phase' is characterized by deep vaginal epithelial cells and a relatively large number of leucocytes. Microscopic bleeding was generally detected. The subsequent 'follicular phase' shows pronounced leucopenia, and the end of this phase is marked by microscopic bleeding, suggestive of an ovulative reaction. It is uncertain whether an ovulation or a follicular involution may possibly occur at this time. The ensuing 'proliferative' and 'premenstrual' phases are comparable to similar phases in normal premenopause smears.

An irregular prolongation of these phases was evidenced in all cases. The longest sexual period observed lasted approximately 5 months.

160. *The modifying effect of old age upon the oestrous cycle in the guinea pig.* George N. Papanicolaou, Department of Anatomy, Cornell University Medical College, New York City.

The sexual rhythm in guinea pigs continues throughout very old age without an indication of discontinuation comparable to 'menopause' in the human. Prolonged anoestrus, when present in old individuals, is due to other disturbing factors.

A general retardation of the sexual rhythm develops as age progresses. In female guinea pigs under one year of age the oestrous cycles average 15½ days in length. After the fifth year of age, the average cycle rises to over 17 days, and after the sixth year, to over 19 days in length. The range of variation, which in young animals is from 14 to 18 days, lengthens to 21, 24, 28, or 31 days in old animals.

Ovulation continues uninterrupted. Even in animals having cystically degenerated ovaries, ovulation occurs and new corpora lutea develop.

Sterility generally appears in old animals, though their oestrous cycle and ovulation still continue and copulation may occur. The disorganization of the sexual cycle resulting from its abnormal prolongation as well as degenerative changes affecting the genitalia may be responsible for the sterility of old age.

161. *Some fiber connections of the tectum of the rat.* James W. Papez, Cornell University, Ithaca.

In a number of Marchi preparations of the midbrain of the rat there are seen, as a result of tectal lesions, degenerated fiber systems which pass from the tectum in a forward direction. So far as can be judged from the preparations, the fibers arise in the tectum. They may be divided into two systems.

1. Fibers from the tectum passing forward to end in the pretectal nucleus and the associated nucleus of the posterior commissure. They probably correspond to the brachium tecti optici of Kappers.

2. From lesions in the pretectal nucleus, especially when the lesion is rather deep, fibers pass forward through the medial medullary lamina and through the area of the centrum medianum, i.e., through the nucleus ventralis pars dorso-medialis of Gurdjian ('27). These fibers are also illustrated by W. E. LeGros Clark ('32, *Philos. Trans.*, fig. 8b rat th 1). They appear to constitute an ascending intralamellar system of fibers probably arising in the tectum and having a more or less definite terminal relation to the medial nucleus of the thalamus. Possibly they are the mammalian equivalent of the tecto-thalamic tracts described in the reptiles by Huber and Crosby ('26 and '33) and others.

162. *The effect of serum on fibroblasts cultivated in vitro.* Raymond C. Parker, Rockefeller Institute for Medical Research. (Introduced by R. G. Harrison.)

Earlier work by Carrel and his associates has shown that serum has a growth retarding effect upon fibroblasts cultivated in vitro. This is due to the antagonistic action of growth-activating and growth-inhibiting substances contained in the serum.

Recent studies, designed to compare the action of serum, heparinized serum, and heparinized plasma on pure strains of fibroblasts, have shown that, under appropriate conditions, the cells are able to overcome the inhibiting effects of serum and plasma and to multiply in them for prolonged periods.

The first effect of these substances is invariably injurious, the degree of injury differing according to the race and medium employed. With the passage of time, however, the colonies undergo gradual improvement, both in the appearance of the component cells and in their rate of proliferation. The adaptability of fibroblasts to adverse conditions manifests itself in time. It is the result of physiological duration.

Serum, as a culture medium, provides a system that is easy to manipulate and one in which fibroblasts remain in good condition, with slow multiplication, for long periods. Fibroblasts from muscle have been cultivated in serum and Tyrode solution for 150 days. In cases where the colonies have been left undisturbed for the last 70 days of this treatment, the cell population has become decidedly heterogeneous in appearance.

163. *The connections of nucleus dorsalis (Clarke's column) in the cat.* I. J. Pass, Department of Anatomy, University of Minnesota. (Introduced by A. T. Rasmussen.)

Examination of the fiber connections of nucleus dorsalis of the cat was undertaken by refined unilateral injection of silver nitrate and distilled water and making small mechanical lesions in and between these nuclei from a dorsal approach. Fine glass pipettes with large bulb on exposed end were used. By electrically heating the air in the bulb, the fluid was expelled. Dorsal roots of various spinal nerves were sectioned. Twenty cats gave results of value. Marchi's method shows that many fibers from dorsal roots of the large nerves supplying the lower extremities ascend into nucleus dorsalis. Few fibers from the midthoracic nerves terminate in this nucleus, which is against the visceral function occasionally ascribed to nucleus dorsalis. No fibers from cervical nerves descend into nucleus dorsalis. Cells in nucleus cuneatus must serve the same proprioceptive function to fibers from the upper extremity that cells of nucleus dorsalis serve to those from the lower extremity. In man, von Monokow's external nucleus (of nucleus cuneatus) which has the same unique cytological characteristics possessed by the cells of nucleus dorsalis (confirmed by study of normal brain stems and spinal cords of man), is probably homologous to nucleus dorsalis. Practically all the fibers arising from nucleus dorsalis cross over to the opposite dorsal spino-cerebellar tract in the cat.

164. *Some effects of anterior pituitary and thyroid secretions on regeneration of the hind leg in Triturus viridescens.* Dorothy Richardson, Department of Zoölogy, Mount Holyoke College. (Introduced by A. E. Adams.)

Injections of phyone (van Dyke and Wallen-Lawrence preparation) accelerate the growth and differentiation of regenerating hind legs in *Triturus viridescens* (A. E. Adams, *Anat. Rec.*, vol. 52, p. 45). To test the possible role of thyroid secretion in this result, similar treatment was given to thyroidectomized newts. Four series of 30 animals each, with right hind leg amputated at midfemur level, were given 0.25 cc. injections every other day, October 17th to February 28th, omitting December 8th to 28th. Measurements of amount regenerated in 4 months follow: phyone-injected normal animals (27 averaged), 6.6 mm.; normal controls (22 averaged), 5.9 mm.; phyone-injected thyroidectomized animals (10 averaged), 5.5 mm.; thyroidectomized controls (8 averaged), 4.5 mm. The average length of the second toe in the same series is 2.3 mm., 1.7 mm., 1.5 mm., and 1.2 mm., respectively.

The contrast between the thyroidectomized and non-thyroidectomized series seems to indicate that regeneration is retarded in the absence of the thyroid, possibly associated with a lowered metabolic rate. Increased growth, however, is obtained in both thyroidectomized and non-thyroidectomized phyone-injected animals, compared with their own controls. Thus the growth hormone of the pituitary seems to exert its effect independently of the thyroid. If differentiation is in part indicated by toe length, it is greatest in the normal phyone-injected animals. This might perhaps be attributed to thyroid hormone.

165. *Observations on the structure and innervation of human digital arteries.* James B. Rogers, Department of Anatomy, University of Louisville School of Medicine.

Histological study of the digital arteries from individuals with vascular diseases emphasized the need for detailed information concerning the structure of normal digital arteries.

The points considered were, 1) age and sex, 2) thickness of each layer of the arterial wall, 3) the amount, type, and distribution of connective tissue, 4) the size of smooth-muscle nuclei and, 5) the innervation of the vessel wall.

The available human material, from persons without apparent vascular disease, was fixed in 10 per cent formalin or Zenker's solution. Sections were stained with connective tissue stains and hemalum. Measurements were made on these preparations. The innervation was demonstrated by silver staining after formalin fixation.

Arteries from five males, ranging from 22 to 35 years of age, were studied. The thickness of the adventitia was variable; the media was approximately one-third of the diameter of the lumen, the intima was from 10 to 15 μ in thickness. The internal elastic membrane was about 4 μ . The external elastic membrane was formed by an increase in the amount of elastic tissue at the periphery of the media. The nuclei of the smooth-muscle cells were 16 to 24 μ in length. There were about 20 layers of circular smooth-muscle cells. In a few preparations fine branching collaterals of non-myelinated nerve fibers were traced close to smooth-muscle cells.

166. *A low viscosity nitro-cellulose for embedding tissues.* Saul Ruby, Department of Anatomy, University of California. (Introduced by Herbert M. Evans.)

Rapid progress has been made in the manufacture of a low viscosity nitro-cellulose due largely to the lacquer industry, and today material may be obtained for histological technique at 30 cents per pound—a low price when compared with that of celloidin, \$1.25 per ounce. Besides cheapness, the great advantage of this nitro-cellulose lies in the rapidity with which it may be put in solution. A 25 per cent solution may be prepared in a few hours, whereas 'celloidin' takes several days.

Tissues may be infiltrated with three concentrations of nitro-cellulose, namely, 6 $\frac{1}{2}$, 12 $\frac{1}{2}$, and 25 per cent. Since 100 gm. of the 'cotton' contain 30 gm. of alcohol, occupying a volume of 37 cc., it is necessary to dissolve the 100 gm. in 103 cc. of alcohol plus 140 cc. of ether to make a 25 per cent solution. The lower concentrations may be made from the 25 per cent solution.

Tissues, following fixation and dehydration, are left 6 to 24 hours in each solution and embedded in the 25 per cent by allowing slow evaporation of the solvents and hardening with chloroform, or by placing immediately into chloroform $\frac{1}{2}$ hour and then into 80 per cent alcohol to be stored until cut.

Acetone may be used as a solvent substitute for the 50:50 ether-alcohol mixture. Stained histologic sections prepared more than a year ago show no deterioration which might be expected were the nitro-cellulose to break down and liberate harmful chemicals.

167. *Homoplastic and heteroplastic transplantation of eyes in Anura.* Joseph L. Schwind, Department of Anatomy, Cornell University Medical College, New York City.

Homoplastic eye transplants were made during larval stages of *Rana sylvatica* and *R. pipiens*. Almost all of the transplanted eyes developed normally and survived metamorphosis. A few, however, degenerated. In favorable cases, the ability to perform coordinated movements reappeared in the transplanted eye 7 days after operation. In some series of experiments as many as 80 per cent of the larvae eventually succeeded in reestablishing ocular movements in the grafted organ. Regeneration of the optic nerve occurred in almost all of the cases examined, but in only half of them did the optic nerve connect with the diencephalon. In the remaining cases it ended free in the connective tissue of the head.

Eyes transplanted heteroplastically between *Rana sylvatica*, *R. palustris*, and *R. pipiens* larvae degenerated within 15 days after operation. Eyes transplanted heteroplastically in embryos of these species, however, differentiated normally, grew for a considerable time, and then suddenly degenerated. So far only one heteroplastic eye, transplanted in the embryonic stage, has continued its growth and survived metamorphosis.

These results, taken together with those previously published on this problem by Born and Harrison, suggest that, with respect to the behavior of heteroplastic grafts, the *Anura* stand midway between urodeles and the higher animals.

168. *Quantitative estimation of the ash in microincinerated preparations.* Gordon H. Scott, Department of Anatomy, Washington University School of Medicine, St. Louis.

By taking advantage of the light refraction of the ash particles in darkfield illumination, it has been possible to devise a method for the quantitative estimation of the residue. With a constant source of light the amount of reflection from particles of like size is roughly proportional to the number of particles in the field. The light intensity from any given area in the microscope field can be measured by means of a gas filled photoelectric cell. Since the amount of light is small, the resulting current set up by the photoelectric cell is too slight to be measured by ordinary means. Therefore, a suitable amplifying device employing an F P 54 tube was constructed which permits readings to be taken directly from a sensitive galvanometer.

The microscopic field is selected and focused sharply on a ground glass. The areas not desired are covered with black paper and the ground glass removed and supplanted with a clear glass. The light from the exposed area is then focused sharply on the sensitive plate of the photoelectric cell by means of a plano-convex lens of short focal length.

The procedure gives an accurate basis of comparison of the ash content of control cells with similar ones which have been subjected to experimental alteration. The resulting figures are expressed in relative, but not absolute terms. (See demonstration no. 32.)

169. *The volume of the neocortex and the changes it undergoes with age.* C. G. Smith, Department of Biology, University of Toronto. (Introduced by E. Horne Craigie.)

The volume of the cortex in the rat, with increasing age, is as follows: at birth 20.04, at 21 days 182.3, at 56 days 201.7, at one year 226.3, at 2 years 205.6, and at 3 years 206.8 cu.mm. Among individuals of the same age the volume varies considerably; at 86 days the range is from 195.8 to 211.1 cu.mm.

The percentage volume of the cortex at birth is 17.11, at 21 days 24.93, after which it decreases to 21.88 per cent at 2 years. At 3 years the value is 22.50 per cent. Among individuals of the same age the percentage volume varies considerably; at 86 days the range is from 23.02 to 24.78 per cent.

The volume of the cortex in the male and the female at 86 days is 203.8 and 202.7 cu.mm., respectively. The percentage volume is greater in the female (25.36) than in the male (23.84).

The number of nerve cells in the cortex at birth is 740×10^4 , and at 4 weeks 1649.0×10^4 . The number falls to 1447.0×10^4 at 15 weeks.

If man develops correspondingly, the volume of his cortex will be at birth 81 cc., at 16 months 228 cc., at $4\frac{1}{2}$ years 271 cc., at 30 years 312 cc., at 60 years 285 cc., and this volume is maintained in old age.

170. *A physiological and histological study of the motor cortex of the dog (*Canis familiaris*).* Wilbur K. Smith, School of Medicine and Dentistry, University of Rochester.

Faradic stimulation of the cerebral cortex of the dog, under ether anaesthesia, showed that the motor area was limited to the cortex around the cruciate sulcus. The hind-leg area occupied the most medial part of the posterior sigmoid gyrus, adjacent to the longitudinal cerebral fissure. More laterally, the cortex responded with fore-leg movements. The movements of the extremities were always contralateral and predominantly flexion in type.

Lateral to the fore-leg area and overlapping its medial edge was a region which upon stimulation caused a turning of the head to the opposite side. In the cortex surrounding the lower end of the cruciate sulcus, stimulation resulted in movements of the facial-masticatory muscles. The facial-masticatory area in its posterior part overlaps the area from which turning of the head may be elicited. Histological examination of the electrically responsive cortex showed that it was characteristically motor in type.

In contrast to the findings of Bechterew and others, the cortex of the medial part of the anterior sigmoid gyrus, adjacent to the longitudinal cerebral fissure, was found to be inexcitable. In some dogs this inexcitable region was found to occupy almost the entire medial half of the anterior sigmoid gyrus. Histological examination of this unresponsive region shows that it has a cyto-architectural structure non-motor in type. It belongs to the area frontalis agranularis of Brodmann.

171. Studies of living nerves. Nerve irritation. Carl Caskey Speidel, Department of Anatomy, University of Virginia Medical School.

The reactions of single internodal segments have been observed in living frog tadpoles, following many varieties of experimental irritation, both of acute and chronic types.

In an irritated segment there is at once initiated a pronounced fluid reaction leading to swelling and vacuole formation. The myelin sheath exhibits a typical rippling and twisting activity, and the axis cylinder breaks away from it in places. It assumes an irregular wavy course and striations (neurofibrils) become visible. The sheath cell nucleus becomes glassy in appearance and is less intimately applied to the myelin sheath. If the irritation is strong, degeneration of the wallerian type follows. If the irritation is relatively slight, the internodal segment may recover within a day or two without undergoing degeneration.

Excellent examples of light chronic irritation have been seen in animals starved for several weeks. Abortive attempts are continually made to repair the lesions in the segments.

Alcohol irritation for a few seconds is sufficient to cause young normal myelin segments to assume a beaded appearance, quite similar to that described by one investigator as the normal condition during myelinogenesis in the human fetus.

Details of the effects on individual fibers have also been noted of burns and scalds, freezing, pressure, cuts, strong anesthetics, acids, and alkalies, endocrine gland extracts, and other agents.

Motion pictures have been obtained, illustrative of some of these irritation phenomena.

172. Studies of living nerves. Polarized light observations on myelinated nerve fibers during growth, regeneration, irritation, and degeneration. Carl Caskey Speidel, Department of Anatomy, University of Virginia Medical School.

Myelin sheath segments, as observed directly in the living organism with polarized light, exhibit characteristic anisotropy of negative character. The neurilemma is isotropic, as are also the axis cylinder and the terminal ameboid growth cones.

Some sheath cells of Schwann contain both isotropic and anisotropic granules. Mesenchymal cells may also contain brilliant anisotropic granules.

There is no evidence, however, that anisotropic granules have any genetic significance as regards myelin sheath origin. On the contrary, the pre-myelin (or young myelin) substance is isotropic.

Prospective myelin sheath segments often may be easily recognized before any condition of anisotropy is apparent. This stage is followed by the appearance of a thin, weakly anisotropic sheath. Later development consists in a gradual increase in thickness and brilliancy of the anisotropic substance, paralleling the growth of the new segment.

A possible chemical basis for this change is the formation of cholesterol ester (which is anisotropic) from cholesterol (which is isotropic).

Irritated fibers exhibit no special changes in the anisotropic condition of the myelin. This remains apparent during the early swelling, rippling, and ovoid formation. Loss of anisotropy occurs in the later stages of degeneration, though in individual globules and granules it may last for some time.

Polarized light motion pictures illustrating these points have been obtained.

173. *Results of transplantation of avian anterior pituitary glands into the newt, Triturus viridescens.* Kathryn F. Stein, Mount Holyoke College. (Introduced by A. E. Adams.)

Transplantation of fresh anterior lobes of fowl pituitary glands (over 1 year of age) into female newts in December stimulated ovulation and egg laying after the administration of 9 glands or less within a period of 7 days. When chick anterior lobes (2 to 10 days after hatching) were used as implants, no ovulation resulted even though as many as 23 glands (2 cases) or 28 glands (2 cases) were implanted within a period of 45 days. Homoplastic transplantations resulted in egg laying within 3 to 11 days. Fourteen glands of young chicks (2 to 10 days) administered over a period of 12 days to hypophysectomized *Triturus* caused molting on the fourteenth day.

Fowl pituitary apparently possesses sufficient hormone to stimulate egg laying in Amphibia; young chick anterior lobes either lack such hormone or the quantity present is too small to be detected by the method or in the number of glands used. The success of young chick anterior lobes in causing molting in the hypophysectomized animal indicates presence of thyroid-stimulating potency, but the thyroid glands have not been examined histologically.

174. *The number of papillae in the kidneys of Primates.* William L. Straus, Jr., Department of Anatomy, Johns Hopkins University.

Certain conflicting claims made in recent years respecting the phylogeny of the Primate kidney have indicated the value of a restudy of this problem. The kidneys of 41 Primates were studied by the writer. In *Tarsius* there was a single papilla in one specimen, several papillae in another. All lemurs studied (2 Lemur, 1 Galago, 2 *Nycticebus*, 1 *Perodicticus*) showed but a single papilla. Of the New World monkeys, *Callithrix* (1 specimen) exhibited but one papilla, *Saimiri* (1 specimen) at least 4, while *Cebus* (3 specimens) and *Ateles* (4 specimens) varied greatly as to the number of papillae, which sometimes differed on opposite sides. Variability was similarly noted in the Old World monkeys, *Pithecius* (5 specimens), *Cercopithecus* (4 specimens), *Papio* (3 specimens), *Erythrocebus* (2 specimens), and *Pygathrix* (1 specimen); in these the two sides sometimes differed, and from 1 to several papillae were present. In 3 *Lasiopyga* 1 papilla was noted. Among the anthropoid apes, the kidneys always showed a single papilla (1 *Hylobates*, 2 *Pongo*, 3 *Pan*). In some specimens classified as having 1 papilla, the pelvic aspect of the medulla was actually concave frontally, without definite papilla formation. The high variability (and frequent asymmetry) of the number of papillae within a single genus leads to the conclusion that this character is of but very limited taxonomic value.

175. *Marchi's staining method: two rapid modifications.* Roy L. Swank, Northwestern University. (Introduced by H. A. Davenport.)

Experiments with tissue of the C. N. S. indicate that oxidation prior to staining is unnecessary. Fixation for 1 or 2 days in 10 per cent neutral formalin was followed by transfer of the tissue blocks, sectioned to 4 mm. thickness or less, without washing, to the staining solution. Two types of staining solutions gave good results. One consisted of Marchi's mixture plus 1½ per cent of glacial acetic

acid, and the other contained potassium chlorate 0.25 per cent, osmic acid 0.33 per cent, and glacial acetic acid 1 per cent. The time of staining for small blocks was about 5 days and for large 8 to 10 days. The chlorate mixture gave very clear backgrounds in the usable peripheral zone of 0.5 to 1 mm. thickness, but diffusely stained centers. The chromate mixture penetrated the entire block. (See demonstration no. 34.)

176. *A statistical study of the hepatic artery and its branches, and of the extra-hepatic biliary ducts.* I. Maclaren Thompson, Departments of Anatomy, McGill University and the University of California.

The arrangements and relationships of the arteries and ducts in the right free border of the lesser omentum have been studied in fifty consecutive dissections of adult human beings of both sexes. The results have been combined with records of similar observations by others. In the case of each anatomical feature, the statistical homogeneity of all the available data has been examined by the chi-square test. Results (if any) whose discordance with the general experience is probably not fortuitous having been discarded, the remainder have been combined into statistical estimates of the occurrence in adults of each anatomical character. The following exemplify the results: in each instance the first figure is the estimated percentage frequency, the appended figure being the standard error of the estimate.

Right hepatic artery from hepatic, 88 ± 1 ; dorsal to common hepatic duct, 78 ± 2 ; dorsal to cystic duct, 13 ± 3 ; dorsal to portal vein, 9 ± 1 . Cystic artery, single, 86 ± 1 ; double, 14 ± 1 ; from right hepatic, 94 ± 1 ; double from right hepatic, 6 ± 1 ; ventral to common hepatic duct, 16 ± 2 ; close to cystic duct, 42 ± 5 . Cystic duct, exhibiting ventral arterial relations, 24 ± 4 ; dorsal arterial relations, 18 ± 4 .

177. *The adolescent growth pattern.* T. Wingate Todd, Western Reserve University.

The occurrence of the menarche corresponds to the registration of a very definite stage of maturation progress, plainly identifiable on roentgenograms of the skeleton and defined on our arbitrary standards (Growth and Development of the Child, The Skeleton, part II, p. 48, White House Conference Reports) as 13 years 9 months. A corresponding stage in male adolescence is registered as an age equivalent of 15 years 3 months. The maximum increment of stature occurs in the months preceding this roentgenographic stage whether in male or female. Physical maturation, however, shows its greatest velocity of increment following this stage.

The patterns of growth as indicated by serial determinations of stature, pelvic breadth, and other anthropometric determinations and of development as betrayed in roentgenographic maturation of the skeleton, give material assistance in elucidating the expression of interplay of the factors promoting adolescence in any particular child under observation and give a reliable scale against which the mental progress and emotional changes may be checked. Clouding of the I. Q. and disturbance of emotional stability are indeed correlated, even in normally developing adolescents, with particular phases of the physical patterns.

178. *On the inheritance of hair weight.* Mildred Trotter and Helen L. Dawson, Department of Anatomy, Washington University, St. Louis.

Given lengths of hair from 208 individuals, comprising 29 families, were weighed. The weight increased with age up to 15 years and decreased somewhat after 50; no sex difference was apparent. A weight-age curve was constructed according to the method of T. R. Running (Trans. Am. Institute of Chem. Engineers, vol. 23, 1929).

The family groups were studied to determine the similarity or difference between parents and children; the hair was judged to be light or heavy according to whether it fell above or below the average indicated by the curve. In general, when both parents have heavy hair the children have heavy or light hair in the proportion of 3 to 1, respectively; when both parents have light hair the children have light or heavy hair in the proportion of 2 to 1, respectively; and when one parent has heavy hair and the other light the children have heavy or light hair in the proportion of 1 to 1.

179. *The normal and experimental development of the mammary gland of the monkey (Macacus rhesus).* C. W. Turner, Department of Dairy Husbandry, and Edgar Allen, Department of Anatomy, University of Missouri.

Mammary glands from normal female monkeys in progressive stages of sexual development have been examined. The prepubertal glands show varying stages of duct growth from a very slight sprouting of the secondary and tertiary ducts to a rather extensive duct system with actively growing end buds.

Monkeys differ from certain other mammals (mouse, rat, rabbit) in that with the recurrence of the menstrual cycle, there develops an extensive lobule system.

Injections of theelin in ovariectomized females followed by short intensive injections of corporin (progestin) induce a slight extension of the duct and lobule system. Extracts of the anterior pituitary (galactin or prolactin), which stimulate lactation in rabbits, have been found to be ineffective in stimulating monkey glands, even where extensive lobule development was present.

Examination of sexually mature males revealed a duct system scarcely extending beyond the base of the nipples. The injection in a normal male of a total of 3,210.5 rat units of theelin during 40 days followed by the injection of 10,900 rat units of theelin in oil during a period of 25 days stimulated growth not only of a considerable duct system, but of extensive lobules as well. A castrate male receiving slightly smaller dosages showed only slight growth. This is the first case, with the possible exception of the guinea pig, that extensive lobule growth has been stimulated with theelin.

180. *The neuroinsular connections in the pancreas of the calf embryo.* Ernest Van Campenhout, University of Montreal.

The study of the pancreas in a series of calf embryos reveals the existence of intimate connections between the primary endocrine islands and the autonomic nervous ganglia.

The primary islands (of Laguesse) arise from the primary pancreatic ducts. Either through a migration of cells toward and in nervous bundles and ganglia, or through an immediate mass contact, they contribute to the formation of 'sympathetico-insular complexes.' The connection between the nervous and the insular elements (serial sections) is more or less intimate; frequently a real interpenetration can be observed. In many instances, the insular elements appear to transform themselves into cells identical to neuroblasts; all the intermediate steps can be found in one complex. Whereas the identification of neuroblasts may at first seem difficult during the young stages of development (7 to 15 cm.), pictures of insulo-neuroblastic transformation are more convincingly observed in the later stages (18 to 25 cm.) when the cytological differentiation of the various types of cells is evident.

The newly formed neuroblastic elements cannot be distinguished from the neuroblasts which have migrated into the pancreas from the coeliac plexus; thus, the intrapancreatic nervous ganglia derive from two sources: neuroectodermal and endodermal.

181. *Topographical relations of cortical lesions to thalamic nuclei in the rat.* W. H. Waller, Cornell University. (Introduced by J. W. Papez.)

Thirty-six lesions were made in the cortex of the white rat. After periods of about 11 days, Nissl staining showed clear areas of degeneration in the thalamus.

Results indicate that all parts of the neocortex receive radiations from the dorsal thalamus, and that the cortex is divided into quite sharply defined areas, with fibers as follows: anteroventral nucleus to entire medial surface of hemisphere, anterodorsal to posterior part of medial surface, anteromedial to anterior part of medial surface, lateral and medial geniculate bodies to medial and lateral parts of posterior cortex, lateral posterior nucleus to a region surrounding the anterior margin of the lateral geniculate area, lateral anterior nucleus to a large area in the dorsomedial part of the cortex, ventromedial and ventral anterior nuclei to the anterior part, ventrolateral nucleus to the region above the rhinal fissure, and ventral nucleus pars arcuata just posterior to the latter.

The centrum medianum and central lateral nuclei are considered as intralamellar nuclei, not related to sharply outlined cortical areas. No cortical radiations were found from the medial, pretectal, or parafascicular nuclei, or from the nuclei of the midline.

A cortical map is presented, and compared with those for the rabbit and cat (von Monakow), and with the cytological maps for rodents (Brodmann, Fortuyn).

182. *On the dark cells in the hepatic lobule of the dog.* Harold L. Weatherford, Harvard Medical School.

Within the hepatic lobule of the dog there are present two types of dark cells. Cells of the first type occur in groups or rows at the periphery of the lobule, being at times especially numerous adjacent to the periportal areas. Following Rumjanzev ('27), cells of this type may be called 'tangential cells' (Heinrichsdorff's cholepoietic cells). The other less interesting type of dark cells may be found singly, or in groups of 2 or 3, any place within the lobule. Marked

differences distinguish the two types. In the tangential cells, chondriosomes appear as very abundant long thin filaments, while in the other type the cell bodies are filled with agglutinated mitochondrial spherules and granules.

Accompanying anaphylactic shock there is a considerable increment of both types of dark cells. Since in the dog the hepatic lesion is a central necrosis, cellular proliferation occurs peripherally and extends centrally. Mitoses are common in the tangential dark cells, some of which are binucleate. From the absence of any degenerative signs, and the presence of numerous well-formed chondriosomes and mitotic figures, it is assumed that the tangential dark cells are very active. At this stage of development, however, there is no evidence that the tangential cells are concerned with bile synthesis. On the contrary, the other type of dark cell has all the appearances of degeneration.

183. Some observations on the spinal accessory and cervical nerves in the innervation of the sternocleidomastoid muscle in the mouse. W. A. Willard and Joseph C. Lawrence, Department of Anatomy, College of Medicine, University of Nebraska.

Complete serial sections were made of this muscle to determine the number and distribution of muscle spindles and character and size of muscle fibers. Further studies were made of the spinal accessory nerve and the anastomosing branch of the second cervical by the osmic and silver methods before and after experimental degeneration. The present study is incomplete in fully separating the distribution of the fibers from these two sources, but the following observations are recorded: 1) The sternocleidomastoid muscle shows a total of 22 to 30 muscle spindles which are not uniformly distributed, but are concentrated more in the finer-fibered divisions of the muscle divisions of the muscle. 2) The spinal accessory nerve to this muscle contained an average of 80 large and medium and 40 very fine medullated nerve fibers, the second cervical branch an average of 35 medium and 45 fine, the latter slightly larger than the fine fibers of the accessory. Unmyelinated fibers are absent in both. 3) The same spindles were shown to be innervated in some cases by fibers from both cervical and accessory. Medium sized fibers from the cervicals were traced to the equatorial part of the spindle and fine fibers from the accessory to the poles of the spindle.

Accepting the former to be afferent and the latter efferent, as has been interpreted, the results do not so far prove a sensory function for the accessory.

184. Neurofibrillar development in the central nervous system of cat embryos between 8 and 12 mm. long. William F. Windle, Anatomical Laboratory, Northwestern University Medical School.

In this series, all afferent nerves are present, but, even in the oldest embryos, the olfactory contains no neurofibrils and the optic and cochlear are less mature than others. Primary sensory pathways are the trigeminal-vestibular tract (containing also facial, glossopharyngeal, and vagus fibers), tractus solitarius, spinal funiculus, and a unisegmental (possibly crossed) vestibular bundle.

All motor nerves arise from a single longitudinal column of neuroblasts; visceral elements lie medial to somatic ones (except in spinal cord). Visceral nuclei migrate laterad to their characteristic positions. Individual neuro-

blasts extend dendrites toward the sensory tracts, elongate, and follow these. Many which have developed stainable processes before migration begins leave their axones anchored in the medial position. In this manner a genu is formed in each of the cranial visceral nerves. The facial nucleus, partially crossed, migrates last; its genu is larger than others. No genu is formed in any of the spinal nerves. Longitudinal shifting of somatic motor nuclei seems to be entirely passive.

An extensive system of association and commissural neuroblasts develops in the lateral wall of the neural tube. Their axones form longitudinal pathways. Descending tracts which appear to be developing in the oldest embryos are: olfactory, juxtaoptic, striothalamic, medial longitudinal, tectospinal, rubrospinal, vestibulospinal, and reticulospinal. Ascending, secondary sensory tracts are formed before descending ones in the caudal part of the brain.

185. *Myelogeny of the cat as related to development of fiber tracts and prenatal behavior patterns.* W. F. Windle, M. W. Fish, and J. E. O'Donnell, Anatomical Laboratory, Northwestern University Medical School.

Myelination begins in the rhombencephalic part of the medial longitudinal fasciculus and intramedullary motor rootlets of the facial nerve of the 80-mm. fetus. At 90 mm., sheaths appear in the hypoglossal, spinal motor, accessory, and trigeminal motor rootlets, the cuneate, descending trigeminal, direct vestibulospinal, and reticulospinal fascicles, the ventral spinal funiculus, and descending mesencephalic component of the medial longitudinal fasciculus. In the 100-mm. fetus, myelination has begun in the vestibular, oculomotor, trochlear, abducens, vagus, and glossopharyngeal nerves, the mesencephalic trigeminal root, tractus solitarius, posterior commissure, trapezoid body, restiform body, tectobulbar, dorsal spinocerebellar, and ventral spinocerebellar tracts. At 110 to 120 mm., the uncinate, vestibulocerebellar, rubrospinal fascicles, and the acoustic stria acquire myelin. The optic, cochlear, and sensory facial nerves are myelinated in the 130-mm. fetus, but few other tracts appear before birth. The number of sheaths in those already mentioned continues to increase, and a secondary trigeminal mechanism may be present at 130 mm.

Sequence of myelination in fiber tracts and intramedullary nerve roots does not always follow the order of development of axones in the embryo as revealed by silver stain. Furthermore, myelination is not always an antecedent of function. The vestibular mechanism receives its sheaths before the vestibular righting reflex appears in 110-mm. fetuses. However, no distinct myelinated pathway, which could transmit impulses mediating the earlier appearing body righting reflex, is present before birth. (See demonstration no. 37.)

186. *The presence of a capillary bed in the umbilical cords of certain mammals.* George B. Wislocki, Harvard Medical School.

It is stated that the tissues of the umbilical cord of the human fetus are avascular. The umbilical and vitelline vessels arise initially from vascular plexuses. These plexuses give rise early to the vitelline vessels (which subsequently degenerate) and to the umbilical vessels. Thus the stroma of the definitive cord is avascular in that it is devoid of a capillary bed.

In the sow the situation is quite different. In pig fetuses ranging from 100 to 250 mm. crown-rump length, I have been able to inject a rich set of blood vessels in the stroma of the cord. One can trace the origin of many minute arteries and veins given off at frequent intervals from the umbilical vessels.

Similar vascularization of the tissue composing the cord throughout all or a great part of the duration of pregnancy can be seen in other mammals.

Mammals which show capillary vascularization of the definitive cord are: pig, hippopotamus, peccary, porpoise, hyrax, sloth, agouti, capybara, Galeopithecus.

Mammals in which the stroma of the fully developed cord is non-vascular are: tapir, Galago, marmoset, howling monkey, spider monkey, gibbon, chimpanzee, and man.

Thus it is seen that outside of the order Primates the majority of mammals possess a capillary circulation in the fully developed cord. The observations may have a bearing on the mode of formation or absorption of the fetal fluids in the different classes of mammals.

187. *On the placentation of the manatee (Manatus latirostris)*. George B. Wislocki, Harvard Medical School.

An excellently preserved fetus (44 cm. snout-tip of tail) in situ with membranes and placenta. The only other descriptions of placentation in the Sirenia are by Harting (1878) and Turner (1879) on two poorly preserved specimens of *Halicore dugong* (fetuses 28 and 163 cm.). According to them, placenta is at first diffuse, later zonary, while limited microscopic examination shows it to be non-deciduous.

In present specimen membranes and placenta occupy one uterine cornu. Placenta is zonary and sharply delimited. Allantois large, filling chorion from pole to pole and leaving only a small triangular area for amniochorionic fusion. Allantois partially subdivided into four large sacs, septal margins of which convey the allantoic vessels to the chorion. There is no yolk sac at this stage.

The placenta on microscopic examination is found to be composed in its entirety of intimately fused chorionic and uterine tissue. A fine-meshed labyrinth is seen which is construed as being of the hemochorial variety. The organization of the placenta in the manatee places this animal definitely in the ranks of the Decidua, and removes it in regard to placentation from any close association with the Cetacea, Artiodactyla, or Perissodactyla. It is possibly most closely allied to the Hyracoidea and Proboscidea, for accounts of placentae of the latter describe them as being zonary and deciduate, although distinctly unlike the endotheliochorial zonary placentae of carnivores.

188. *Cyclic histological variations in the cell types found in the anterior hypophysis of various species*. J. M. Wolfe and Rucker Cleveland, Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine.

In the anterior pituitaries of dogs, sows, rats, and rabbits there are cyclic histological changes in the anterior hypophysis which are correlated with the different phases of the oestral cycle. In dogs, sows, and rabbits these variations are both qualitative and quantitative; in rats (unmated cycle) they are qualitative. In dogs, sows, and rabbits, during the lutein phase, the cells of type I decrease

in relative percentage and show evidence of granular loss, while the relative percentage of the cells of type IV increases. Induction of a lutein phase in the rat (pregnancy or pseudopregnancy) brings about similar changes in these cells (type I). In all species studied during prooestrus (except rabbits), the cells of type III are filled with granules. In oestrus (including rabbits) the granular content of the cells is markedly reduced. There is a definite quantitative relation between the granular and non-granular forms of this type (type III) during these two phases of the cycle. In all species the granular cells reach a minimum during the lutein phase. There is a parallel increase in the type IV cells. Cell counts have been made on every fourth or fifth slide from complete serial sections of pituitaries of 275 animals. 1,548,457 individual cells have been counted. (See demonstration no. 39.)

189. Changes in the anterior hypophyses of rats subjected to extreme partial castration. J. M. Wolfe, Doris Phelps, and Rucker Cleveland, Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine.

The oestral cycle of rats subjected to extreme partial castration is extremely variable. These animals may exhibit many prolonged oestral periods, so that by the end of the observation period they have been in a full oestrus for as much as 46 per cent of the time. The uterine glands of such animals often exhibit cyclic dilation. Other instances occur in which these animals only rarely come into oestrus. An animal showing a hyperoestral condition may later pass into a hypooestral phase. In the anterior hypophyses of these animals no definite changes are found in the cells of type I (eosinophiles), but the cells of type III (basophiles) vary directly, both quantitatively and qualitatively, with the type of oestral cycle exhibited. In animals in which the hyperoestral condition is extreme, the cells of type III show reduction both in relative percentage and granular content. In animals which only occasionally come into oestrus (10 per cent of observation period of 100 days), the relative percentage of type III (packed with granules) is high. Many castration cells are also present (as high as 4.7 per cent). There are intermediate conditions between these two extremes. (See demonstration no. 39.)

DEMONSTRATIONS

1. *Film showing capillary circulation and contraction of arterioles, as seen in transparent chambers inserted in the rabbit's ear.* E. R. Clark, Eleanor Linton Clark, and E. A. Swenson, Department of Anatomy, University of Pennsylvania.
2. *The beginning of peristaltic contraction in the embryonic chick heart.* (Motion picture.) Bradley M. Patten, Western Reserve University. See abstract no. 71.)
3. *The thyroid stimulating potency of phyone and hebin.* A. Elizabeth Adams, Zoölogy Department, Mount Holyoke College. (See abstract no. 95.)
4. *Pigmentation of meninges in Norway rats.* W. H. F. Addison and Doris A. Fraser, Department of Anatomy, University of Pennsylvania. (See abstract no. 3.)
5. *Phonographic records of auditory impulses from the dog's ear.* T. H. Bast, J. A. Eyster, R. West, O. L. Backus, R. Noer, and R. Krasno, University of Wisconsin.
6. *The emptying of the gall bladder in children and monkeys.* Edward A. Boyden, Institute of Anatomy, University of Minnesota. (See abstract no. 35.)
7. *Golgi apparatus in acute and chronic morphinism.* A. R. Buchanan, Department of Anatomy, State University of Iowa. (Introduced by E. M. MacEwen.) (See abstract no. 146.)
8. *Induced maturity of the reproductive tract of salamanders through the agency of the gonad stimulating principle of the anterior hypophysis.* R. K. Burns, Jr., and Adrian Buyse, School of Medicine and Dentistry, University of Rochester. (See abstract no. 104.)
9. *The effects of x-radiation on the regeneration of the fore limb of *Amblystoma* larvae.* E. G. Butler, Laboratory of Comparative Anatomy, Princeton University. (See abstract no. 19.)
10. *Microscopic preparations showing the behavior of the epiphyseal cartilage in rachitic rats.* G. S. Dodds, Medical School, and H. C. Cameron, Agricultural Experiment Station, West Virginia University. (See abstract no. 29.)
11. *The stainability of Nissl material in adrenalectomized rats.* J. C. Donaldson and Ira D. Hogg, Department of Anatomy, University of Pittsburgh School of Medicine. (See abstract no. 121.)
12. *Specimens of bones of the foot illustrating primitive elements in man.* Thomas Horace Evans, Long Island College of Medicine. (See abstracts nos. 33, 124, 125, 126.)
13. *A new method of coloring lantern slides from photomicrographs.* Charles Goosman, Cincinnati.
14. *Cellular constituents of the anterior pituitary after uterine implants of carcinoma in rats.* M. F. Guyer and P. E. Claus, University of Wisconsin. (See abstract no. 37.)
15. *A method of transplanting limb buds in chick embryos.* V. Hamburger (Rockefeller Foundation Fellow), Hull Zoölogical Laboratory, University of Chicago. (Introduced by B. H. Willier.) (See abstract no. 131.)
16. *The state of development of the central nervous system of a 16-mm. human embryo, Homo no. 1, Pittsburgh, 1932.* Ira D. Hogg, Department of Anatomy, University of Pittsburgh School of Medicine. (See abstract no. 42.)
17. *Human fetal activity.* Davenport Hooker, Department of Anatomy, University of Pittsburgh School of Medicine. (See abstract no. 43.)

18. *Coeliac ganglion cells in the rat and the rabbit.* Everett H. Ingersoll, Department of Anatomy, Medical College of Virginia. (Introduced by H. L. Osterud.) (See abstract no. 46.)
19. *The gross and histological changes produced in the eyes of the progeny of the albino rat when irradiated in utero.* T. T. Job and Nathan Falk, Department of Anatomy, Loyola University School of Medicine.
20. *Specimen illustrating persistence of the left ischiadic artery in man.* Thesle T. Job, Department of Anatomy, Loyola University School of Medicine. (See abstract no. 138.)
21. *Experimental production of internal hydrocephalus by irradiation in utero.* Thesle T. Job, Department of Anatomy, Loyola University School of Medicine. (See abstract no. 137.)
22. *The stomach contents of a cadaver.* Homer B. Latimer, Department of Anatomy, University of Kansas. (See abstract no. 142.)
23. *A drawing of a supraclavicularis proprius muscle.* Homer B. Latimer, Department of Anatomy, University of Kansas. (See abstract no. 141.)
24. *Cerebro-ocular syndromes.* Donald J. Lyle, Department of Anatomy, University of Cincinnati.
25. *Charts showing the variability of the origin of the mylohyoid muscle, the relation of the lingual nerve to the posterior border of this muscle, and the relation of the mylohyoid portion of the superior constrictor muscle at its origin to the mylohyoid.* Shirley P. Miller and H. L. Harris, Department of Anatomy, University of Minnesota.
26. *The cerebrospinal fluid, and the cervical lymph nodes in the living dog.* O. A. Mortensen and W. E. Sullivan, University of Wisconsin. (See abstract no. 65.)
27. *Implantation, fetal membranes and placentation in the squirrels (Sciuridae).* H. W. Mossman, Department of Anatomy, University of Wisconsin. (See abstracts nos. 154, 156, and 66.)
28. *Changes in the testes and accessory glands of the gray and fox squirrel (Sciurus carolinensis and niger) during their seasonal reproductive cycle.* H. W. Mossman, A. M. Katz, and L. H. Rubnitz, Department of Anatomy, University of Wisconsin. (See abstract no. 66.)
29. *Some sections of the human ovary.* D. S. Pankratz, Department of Embryology and Histology, University of Tennessee College of Medicine. (Introduced by C. S. Simkins.)
30. *Sections showing the structure of the vagus nerve of the cat after degeneration of the motor fibers following intracranial section of the vago-accessory rootlets.* S. W. Ranson, Institute of Neurology, Northwestern University Medical School. (See abstract no. 72.)
31. *The activity of the peritoneal epithelium of the testis of the musk turtle, Sternotherus odoratus (Latreille).* Paul L. Risley, Departments of Zoölogy and Anatomy, University of Iowa. (Introduced by Emil Witschi.)
32. *Demonstration of microincinerated preparations of mammalian tissues.* Gordon H. Scott, Department of Anatomy, Washington University School of Medicine, St. Louis. (See abstract no. 168.)
33. *Reconstruction of the arteries, veins, lymphatics, and nerves of a 5 by 6-mm. area from the distal portion of the adult human omentum (30 years).* Parke H. Simer, Department of Anatomy, College of Medicine, University of Illinois. (Introduced by Otto F. Kampmeier.)

34. *Marchi's staining method: two rapid modifications.* Roy L. Swank, Northwestern University. (Introduced by H. A. Davenport.) (See abstract no. 175.)
35. *Comparison of the normal development of the mammary gland with that induced by the successive stimulation of the rudimentary gland with theelin, theelin and corporin (progestin), and galactin (prolactin) in the monkey, rabbit, guinea pig, rat, and mouse.* C. W. Turner, Department of Dairy Husbandry, University of Missouri. (Introduced by Edgar Allen.)
36. *Embryos of the chick, rat, cat, dog, and man prepared by the pyridine silver method.* William F. Windle, Anatomical Laboratory, Northwestern University Medical School.
37. *Myelogeny of the cat as related to development of fiber tracts and prenatal behavior patterns.* W. F. Windle, M. W. Fish, and J. E. O'Donnell, Anatomical Laboratory, Northwestern University Medical School. (See abstract no. 185.)
38. *Demonstration of amphibian parabiotic twins.* Emil Witschi, University of Iowa.
39. *Cell types found in the anterior hypophyses of various species.* J. M. Wolfe and Rucker Cleveland, Departments of Anatomy and Gynecology, Vanderbilt University School of Medicine. (See abstracts nos. 188, 92.)
40. *Reconstruction of the major portion of the vascular channels in an 8.5-mm. opossum embryo, with special consideration of the lymphatic system.* Arnold A. Zimmermann, Department of Anatomy, College of Medicine, University of Illinois.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

PIERSOL'S NORMAL HISTOLOGY, with Special Reference to the Structure of the Human Body. Fifteenth Edition. Edited by W. H. F. Addison. 1932. Size, $6\frac{1}{2} \times 9\frac{1}{4}$ inches. Pages x + 478. 435 illustrations, 43 of which are in color. Index. List of References. Cloth. Price, \$6.00. J. B. Lippincott Company, Philadelphia, Pa.

An excellent textbook has been made better and more up-to-date in this revision. Certain chapters have been rewritten to include important recent discoveries bearing on human histology, namely, those on nerve-endings, glands, biliary passages, the epithelium of the male urethra, and cyclic changes in the endometrium. Both the text and the illustrations display a pleasing clarity of detail. Following the same plan as formerly, this course supplies a basis for work in pathology, familiarizing the student with conspicuous features and characteristic structures of normal human tissues, and giving particular attention to those tissues and organs usually taken at autopsies. Methods employed in the study of cells, such as tissue culture in vitro, microdissection, supravital staining of blood, fixation and staining, are described briefly in the appendix.

NEUROPATHOLOGY, The Anatomical Foundation of Nervous Disease, by Walter Freeman. 1933. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. 349 pages. 116 illustrations. Glossary. Lists of References. Index. Cloth. Price, \$4.00. W. B. Saunders Company, Philadelphia.

A summary of our present knowledge of the changes of the nervous system in diseases. The illustrations and descriptions of conditions characteristic of certain diseases are highly valuable to the pathologist in interpreting what he finds upon opening the cranial cavity or examining prepared sections, and to the neurologist in visualizing the disease processes that are evolving in his patients. A general discussion of cytology and cytopathology of nerve tissues introduces the main body of the book which deals with the specific changes occurring in diseases which affect the nervous system. They include, among many others, arteriosclerosis, meningitis, inflammation of the brain, tuberculosis, syphilis, leprosy, functional psychoses, and intoxications. A list of references for further reading follows each chapter.

MORRIS' HUMAN ANATOMY, A Complete Systematic Treatise. Edited by C. M. Jackson. Ninth Edition. 1933. Size, $7 \times 10\frac{1}{4}$ inches. Pages xiv + 1481. 1166 illustrations, 514 of which are in colors. Index. Lists of References. Fabrikoid. Price, \$10.00. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

The Ninth Edition has been thoroughly revised and some sections have been rewritten, making it completely up-to-date and authentic. In the revision, many new pictures have been added to supplement and amplify the text. In addition

NEW BOOKS AND PUBLICATIONS

to B. N. A. terms, which have been used as formerly, names recommended by the Nomenklatur-Kommission have been added in parentheses. The plan, which proved so satisfactory in the last edition, of printing important and fundamental features of each topic in larger type and details for reference in smaller type, is continued. The editor has coordinated the entire work to insure continuity and completeness, although each author is responsible for his own section or sections. The contents and contributors are as follows: Introduction, by C. M. Jackson. Developmental Anatomy, by R. E. Scammon. The Skin and Mammary Glands, by Charles R. Stockard. Osteology, by Robert J. Terry. The Articulations, by Robert J. Terry. The Musculature, by C. R. Bardeen. The Blood-Vascular System, by Harold D. Senior. The Lymphatic System, by Eliot R. Clark. The Nervous System, by Irving Hardesty. The Special Sense Organs, by Leslie B. Arey. The Digestive System, by C. M. Jackson. The Respiratory System, by J. Parsons Schaeffer. The Urogenital System, by Franklin P. Johnson. The Glands of Internal Secretion, by J. F. Gudernatsch.

OSTÉOLOGIE. I. Membre Thoracique. Membre Abdominal, by A. Hovelacque. 1933. Size, $7\frac{1}{4} \times 10$ inches. 253 pages. 169 illustrations. Index. Price, 60 francs. Gaston Doin et Cie, Paris.

The bones of the upper and the lower limbs are studied as to their shape, structure, and location. The illustrations, drawn by d'Arnoult Moreaux, show the attachments of the more important muscles as well as the appearance of the bones.

THE ACTION OF THE LIVING CELL. Experimental Research in Biology, by Fenton B. Turck. 1933. Size, $6 \times 8\frac{1}{4}$ inches. Pages xi + 308. 17 illustrations. Bibliography. Index. Cloth. Price, \$3.50. The Macmillan Company, New York.

Investigation into the nature of so-called shock in animals has led the author to certain conclusions and theories which he outlines in this book. As a result of his experiments covering 40 years or more, he believes shock is due primarily to the liberation from injured tissue of a toxic entity which he terms cytost. Healthy cells seem to produce in response to small injections of the toxin a protective substance anti-cytost. Minute quantities of homologous cytost exert a distinctly stimulating effect on the cells, while its characteristic toxic action becomes manifest if its concentration is too high, indicating that a certain optimal concentration of cytost is compatible with normal functioning of cells, whereas when this optimum is exceeded in one direction or the other anomalous behavior results. When such anomalous behavior becomes excessive a pathological condition ensues. Further examination of cytost and anti-cytost may lead to understanding of the connection between foci of infection and diseases of obscure origin, such as nephritis or arthritis.

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NOTICES BY F. E. WELLS

BIOLOGY, An Introduction to the Study of Life, by H. Munro Fox. 1932. Size, $5 \times 7\frac{1}{2}$ inches. Pages xv + 344. 152 illustrations. Index. Cloth. Price, \$1.75. The Macmillan Company, New York.

The plan chosen for this book begins with man and ends with fossils, going through the range of animals and plants between these extremes. It is designed as a textbook for students preparing to take the English school certificate examination, but it may also serve as an outline of biology for any reader interested in the subject. Technical terms have been reduced to a minimum and the practical side has been emphasized. The student is encouraged to make observations and studies on animals and plants in their natural habitat. A list of suggestions following each chapter offers the teacher practical instructions for laboratory experiments and demonstrations. The last two chapters are on evolution and the history of biology. An appendix contains a list of Greek and Latin terms used in biology.

THE MECHANISM OF CREATIVE EVOLUTION, by C. C. Hurst. 1932. Size, $7\frac{1}{2} \times 10$ inches. Pages xxi + 365. 200 illustrations. Index. Buckram. Price, \$6.00. The Macmillan Company, New York.

This volume points out the salient features of recent genetical discoveries and shows that the mechanism of the chromosomes, and the genes therein, is the mechanism of creative evolution. The discovery of the genes, the establishment of the principle of mutation, and the experimental determination of the location and linkage of genes of *Drosophila* led to four important advances made in the last 10 years. 1) The discovery of the transmutations of chromosomes and genes, leading to the experimental creation of new varieties and species. 2) The experimental creation of gene mutations and chromosome transmutations by the application of x-rays and other short-wave radiations. 3) The discovery that specific and higher group characters are also represented by genes which, after mutation, behave as mendelian varietal characters. 4) The translation of the Linnean concept of species into terms of genes and chromosomes, thus establishing, in the genetical species, a measurable unit of evolution together with a new exact science of taxonomy founded on an experimental basis. Facts and theories are summarized in the last chapter, where the author views the work done and speculates on further developments and interpretations.

DEVELOPMENT OF AN INFANT CHIMPANZEE DURING HER FIRST YEAR, by Carlyle F. Jacobsen, Marion M. Jacobsen, and Joseph G. Yoshioka. (Comparative Psychology Monograph Number 41.) 1932. Size, $6\frac{1}{2} \times 10$ inches. 94 pages. Illustrated with charts and photographs. Index. Bibliography. Price, \$1.50. The Johns Hopkins Press, Baltimore.

Alpha, the first chimpanzee born in the Yale Anthropoid Experiment Station in Florida, was a bottle-fed baby. Due to the illness and death of the mother

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at the time of the infant's birth, it was necessary to rear Alpha in the laboratory nursery, isolated from her kind, where her bodily and behavioral development was painstakingly and thoroughly studied. Her care, reactions, and abilities are discussed in this monograph and compared with those of the average human infant of the same age.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN, Lieferung 397. Edited by Emil Abderhalden. Abt. VII. Methoden der experimentellen morphologischen Forschung, Teil 1, Heft 9. Experimentelle Morphologie. 1932. Size, 7 × 10 inches. 118 pages. Price, RM 6.

Contains four articles, as follows: Die histologischen Untersuchungsmethoden für die männlichen Geschlechtsorgane, by Friedrich Matzdorff. Über die histologischen Färbemethoden der Hypophyse, by Walther Benoit. Über die histologischen Färbemethoden der Zirbel, by Walther Benoit. Die experimentelle Prüfung der Schilddrüsenfunktion, by Fritz de Quervain and Isaak Abelin.

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A MANUAL OF EMBRYOLOGY, The Development of the Human Body, by J. Ernest Frazer. 1932. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. Pages viii + 486. 282 illustrations. Index. Cloth. Price, \$8.00. The Williams & Wilkins Company, Baltimore.

In preparing this book, Doctor Frazer has preferred to build up for the student a connected picture of the developing embryo rather than a patchy series of discrete descriptions of formation of organs, and has used the regional method of study. For a period of 25 years he has carried on his investigations in embryology and the present volume brings together the results and conclusions of his research. There is no list of bibliographic references; but throughout the text, any statement of theory not generally accepted or not supported by Doctor Frazer's own investigations is followed by the name of its author. The material is divided into two parts. Part One, on early and general development, includes descriptions of the sex cells, fertilization, embedding, segmentation, and structures of intrauterine life. Part Two tells in more detail the growth and differentiation of organs and systems in all regions.

THE RACES OF MAN, DIFFERENTIATION AND DISPERSAL OF MAN, by Robert Bennett Bean. 1932. Size, 6×9 inches. Pages vi + 134. Index. Glossary. Illustrated. The University Society, Inc., New York.

An elementary survey of physical anthropology written in popular and interesting style. It gives a brief review of prehistoric peoples; tells something of the steps in man's ascent and his differentiation into races; traces the more important causes and routes of race dispersal, and points out the distinguishing characteristics of the races and sub-races into which modern man has been divided.

FINAL REPORT OF THE COMMISSION ON MEDICAL EDUCATION, 1932. Size, $6 \times 9\frac{1}{2}$ inches. 560 pages. Index. Cloth. Office of the Director of Study, New York.

The purpose of this study is stated as follows in the Foreword: The Commission on Medical Education was organized in 1925 by the Association of American Medical Colleges to make a study of the educational principles involved in medical education and licensure, and to make suggestions which would bring them into more satisfactory relationships with the newer conceptions and methods of university education, on the one hand, and with the needs of present-day society, on the other. It was believed that such a study would assist the efforts to develop a program adapted particularly to the educational, economic, and social conditions in this country.

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The problems discussed include the public aspects of medicine, medical needs, the supply and distribution of physicians, postgraduate medical education, the internship, medical licensure, the medical course, opinions regarding the medical training, premedical education, the cost of medical education, medical education in Europe. The findings of the Commission are briefly summarized in the concluding chapter. The appendix contains 161 tables of statistical material.

ARBEITEN AUS DEM NEUROLOGISCHEN INSTITUTE AN DER WIENER UNIVERSITÄT. XXXIV Band. 1932. Size, 7×10 inches. 307 pages. Illustrated with 88 text figures. Price RM 60.00. Franz Deuticke, Wien and Leipzig.

In addition to fourteen papers treating as many problems of neurological research, this volume contains a history of the Institute and its growth during fifty years (1882-1932), written by the present director, Prof. Dr. Otto Marburg.

METHODS AND PROBLEMS OF MEDICAL EDUCATION (Twenty-first Series). 1932. Size, 8×10 inches. 226 pages. Illustrated. Index. The Rockefeller Foundation, New York.

The twenty-first volume completes a series, begun in 1924, in which The Rockefeller Foundation has published information regarding teaching methods and facilities in medical schools all over the world. The final volume is devoted to nursing schools and contains descriptions of seven organizations in the United States, three in Canada, and one each in China, Denmark, England, Finland, France, Hungary, and Siam.

VERHANDLUNGEN DER DEUTSCHEN GESELLSCHAFT FÜR KREISLAUFFORSCHUNG. V. Tagung. 1932. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages xix + 362. Illustrated with 95 text figures and 1 plate. Price RM 20.00. Theodor Steinkopf, Dresden and Leipzig.

The fifth session of the Society was held in March, 1932, in Tübingen. This volume contains programs of meetings and text of all papers presented.

ÜBER EPITHELIALE UND MESODERMALE SCHLEIMBILDUNG IN IHRER BEZIEHUNG ZUR SCHLEIMIGEN METAMORPHOSE UND SCHLEIMIGEN DEGENERATION, zugleich ein Beitrag zur Orthologie und Pathologie des Mesenchyms, by Erich Letterer. 1932. Size, $5\frac{1}{4} \times 8\frac{1}{2}$ inches. 59 pages. 21 illustrations. Bibliography. Price RM 7.00. S. Hirzel, Leipzig.

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